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The Preparation of Fresh-fillets of Fish for Smoking

By D. LeB. Cooper and E. P. Linton
Atlantic Fisheries Experimental Station

(*Received for publication June 18, 1936*)

ABSTRACT

Controlled experiments on the variables affecting the pre-treatment of fresh fillets for smoking show that the preliminary ice storage of the fish should be for longer than two days and the fillets should be treated with 80 per cent saturated brine at a temperature of between 1 and 7°C. for approximately 15 minutes. Drying should be with air at a velocity of between 125 and 400 centimetres per second, a wet-bulb temperature between 15 and 23°C. and a relatively humidity below 70 per cent. These conditions must be adjusted so that the drying time is at least from 35 to 40 minutes.

The current method of manufacture of smoked fillets follows closely the technique used in the preparation of "Finnan Haddie". This adherence to the older procedure, together with modifications made expedient by improvements in marketing methods, developed a consumer demand for an article with the following general characteristics: a smooth glossy surface, a characteristic smoky taste, and an evenly distributed golden colour.

Experience and investigation have shown that these characteristics are obtained most easily when the treatment is divided into two parts; a pre-treatment of the fillet during which they are brined and partially dried, and a subsequent smoking; the former produces the characteristic smooth, glossy surface, the latter, the taste and colour. This communication is concerned with the pre-treatment; smoking will be discussed in a later paper.

The subject is discussed in the following order: the effect of changes in drying methods, the influences of variations in the methods of salting, and the determination of limiting conditions for commercial units. (Drying is examined before brining for the effect of change in this variable had not hitherto been determined.).

DRYING

Discussion

It is clear that the word "drying" as applied to this problem may be misleading, for maximum rate of drying is not a necessity; rather it is required to ascertain which set of conditions will produce suitable surface characteristics with the minimum moisture loss. Therefore, the theoretical and practical deductions from drying material other than fish can serve only as a guide. The work of Sherwood (1929), Walker (1927) and Griffiths (1932) is particularly useful in this connection.

A surface which is suitable is one which is smooth and shiny, and should

remain so under normal conditions of handling, and should smoke evenly. These requirements are satisfied if treatment is carried to the stage where the surface becomes tacky, and is not broken by light friction.

Experimental

Similarly treated cod fillets were suspended in a duct through which air at known conditions was passed. The fish were hung so that all faces were parallel to the direction of air flow.

The drying tunnel was 22.8 cm. square and 304 cm. long. The arrangement is shown in figure 1. Air humidities were controlled by an air washer and suitable heaters. Air speeds were set by the use of blast gates, and measured by a standard pitot-static tube (E) at high velocities, and the shaped outlet nozzle (F) at low velocities. At intermediate speeds, values obtained from both instruments were compared, and checked against independently calibrated hot-wire anemometers. The distribution of velocities at the point of suspension (J) was normal. Temperatures were read on the compared mercury thermometers at (G).

The calculated precisions are: air velocities, 3 per cent; air temperatures, 0.1°C.; humidities, 2 per cent.

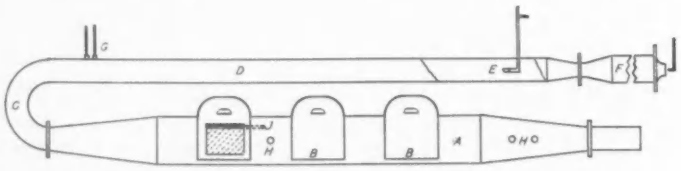

Figure 1. Diagram of experimental tunnel showing measurement systems. A—tunnel, B—observation door, C—radius bends, D—circular duct, E—pitot-static tube, F—chamber and shaped nozzle, G—thermometers, H—thermometers, J—tenter.

Examination for the effects of drying consisted of frequent inspection of the suspended fillet to ascertain the interval required to cause the cut surface to become tacky. This condition was detected by touching the surface. The interval is called "time to dryness". At the latter point the fish were removed from the tunnel and examined for sheen, smoothness, and stability of surface; the first two by comparison with an undried fish under north light, the third by the effect of light rubbing which causes separation and rewetting of an unstable surface.

In recording the results, five grades were distinguished for each feature of the surface—sheen, smoothness and stability,—and these grades have been indicated by the numbers 0, 1, 2, 3 and 4. The untreated fillet used for comparison is represented by 2. Grades with numbers greater than 2 indicate an increase in the feature measured, and those with numbers less than 2 a decrease. The approximate error was found by trial to be about 25 per cent.

These methods of examination are open to the objection that they depend entirely on personal judgment. They are, however, sufficiently precise, since the variations in the raw material are of the same magnitude as the error in personal judgment.

The experimental details may be followed from the following data for a typical run (No. 77).

Fillets, freshly cut from winter-caught unspawned cod-fish, which had been stored in ice for four days after killing, were placed in a solution of 60 per cent saturated brine at 9°C. for 20 minutes. These were removed, drained for 3 minutes and hung for treatment. Fillets treated in this manner are called "standard fillets". Preliminary experiments showed that more careful treatment was unnecessary, for the two fillets from one fish showed as great differences as similarly treated fillets from separate fish. The results were as follows:

Weight changes

Weight of fresh fillet..... 248.5 gm.
Weight of brined fillet..... 261.2 gm.
Weight of dried fillet..... 248.7 gm.

Air temp. entering	Times	Velocity press mm. water	Leaving air D.B. °C.	Leaving air W.B. °C.	Surface Dryness	Surface Sheen	Water in washer
30.2	10.24	0.49	30.4	18.2			11.9
	10.33				Wet		
30.0	10.40	0.49	30.2	18.3	Drier	4	12.5
	10.44				Slight tacky		
	10.46				Normal		10.8

Remarks. Surface stability 3. Surface smooth.

TABLE I. The effect on sheen and surface stability of drying to a tacky surface with varying velocities and temperatures of the air and with different dew-points.

With dew-point of 5°C.																				
Air velocity (cm./sec.)	265					185					105					55				
Air temperature (°C.)	40	35	30	35	20	40	35	30	25	20	40	35	30	25	20	40	35	30		
Sheen	1	1	2	2	3	0	0	2	3	4	1	2	3	4	4	3	2	3		
Surface	0	1	2	2	3	0	0	1	2	4	1	3	4	4+	4+	–	–	–		
Time to tacky surface (minutes)	9	11	12	16	21	11	13	14	18	18	15	20	31	35	52	25	27	39		
With dew-point of 11°C.																				
Air velocity (cm./sec.)	265					180					110									
Air temperature (°C.)	35	30	25	20		40	35	30	25	20	40	35	30	25						
Sheen	2	2	3	3		2	3	4	4	3	2	3	3	4						
Surface	0	1	2	3		0	3	2	3	4	0	2	3	4+						
Time to tacky surface (minutes)	9	17	25	30		10	18	22	29	42	15	23	30	36						
With dew-point of 17°C.																				
Air velocity (cm./sec.)	260					200					105					60				
Air temperature (°C.)	40	35	30			40	35	30			40	35	30	25		40	35	30		
Sheen	2	2	3			1	2	3			1	2	3	3		2	3	3		
Surface	–	2	3			0	2	3			3	3	4+	3+		3	4	4+		
Time to tacky surface (minutes)	17	19	26			16	23	26			26	30	35	50		38	51	56		

RESULTS

The results of 96 experiments are summarized in table I. The values given are means of two or more determinations. Individual weight changes are not shown. Mean weight changes are as follows:—gain during brining 3.85 (Average Deviation 0.86) per cent of the fresh weight; loss during drying 3.66 (A.D. 0.76) per cent. It appears therefore that if tacky surface be taken to be the criterion for the degree of dryness, the per cent weight loss is nearly independent of the actual drying conditions within the range of air conditions studied.

The results of the determinations have been plotted and are shown in figures 2, 3, and 4.

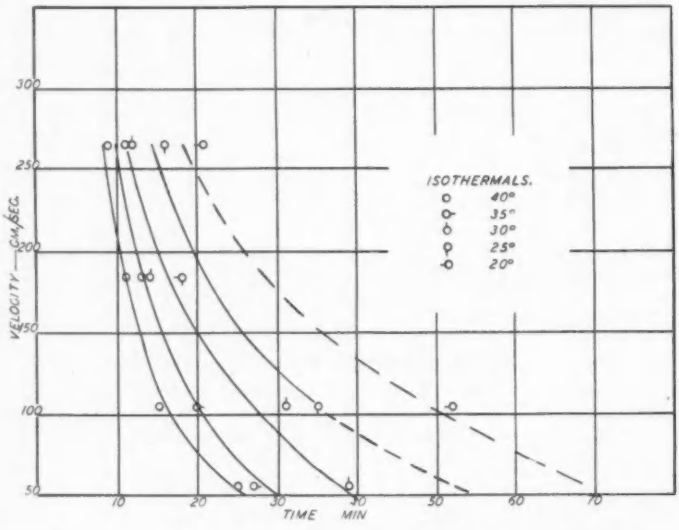

FIGURE 2. Change in time of drying to produce tacky surface. Dew-point constant at 5°C.

DISCUSSION OF RESULTS

Lightly salted fish muscle may dry with a skin effect (Weld unpub.). The conditions such that a skin will form are (1) that the chemical and physical characteristics of the sample will permit such a formation, and (2) that the conditions used in drying are suitable for the production of a skin. The skin is a layer of definite boundary formed first at the surface of the sample. The moisture gradient in this layer is different from that in the main body of the sample during drying, and its water concentration less. In the case of fish muscle the skin will not return to its original condition after soaking (Cloake 1924).

The criterion of tacky surface is a rough measure of the concentration of water in a thin layer at the surface. This is

$$\frac{\partial c}{\partial t} = D - E$$

where c is the concentration in the layer, t is the time, D the rate of diffusion into the layer, and E the rate of evaporation from the layer. D is a function of c, $\frac{\partial c}{\partial x}$ and $\frac{\partial^2 c}{\partial x^2}$. At the commencement of drying when the surface is uniformly wet with a layer of liquid phase the rate of evaporation may be expressed by

$$E = K\ (P_s - P_a)\ (1 + bu^{0.85})\ \text{(Griffiths 1932)}$$

where k is a constant, a function of the size, shape and orientation of the fillet, P_s is the vapour pressure at the surface, P_a the vapour pressure of water in the air, B a constant and u the air speed.

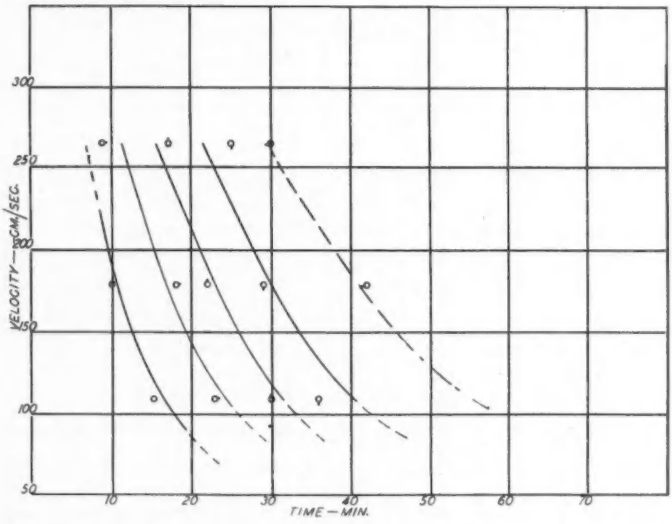

FIGURE 3. Change in time of drying to produce tacky surface. Dew-point constant at 11°C.

E is greatly affected by external conditions, D not so much so; clearly then, conditions may be so arranged that E is $>$, $=$ or $<D$. Thus the thickness of the skin formed is a function of $D-E$. Also, at the commencement of drying if E be large compared to D then the surface appears dry in a short time and a thin skin is formed; if E be equal to D, it will become greater than D as drying proceeds, hence a skin will form which will be thicker than that in case above. If E be kept less than D during the whole process, than clearly no skin will form and the sample will dry nearly uniformly throughout. Under conditions nearing the latter, the fillet becomes toughened.

Two clearly defined effects, noticed during experimental drying, tend to substantiate this explanation. When the fillet was dried under conditions in which E was large the surface became tacky in a clearly defined short time. The skin was thin and unstable. Under such conditions that D was approximately the same as above (equal wet-bulb temperatures) but E greatly lowered, the time

to dryness was large and the interval poorly defined. The skin was thick, and in extreme cases the fillet very tough.

The above discussion leads to the supposition that there exists some relation between the values of D and E within the range studied at which the surface layer would be just thick enough to be stable. This presupposes a minimum time of drying. An average of experimental results substantiates this and shows that the minimum time of drying is between 30 and 35 minutes. If drying to a tacky surface be completed within a shorter time the surface is unstable and may assume an unpleasing wrinkled appearance. The following was therefore chosen as standard drying procedures for further testing of other variables; such conditions

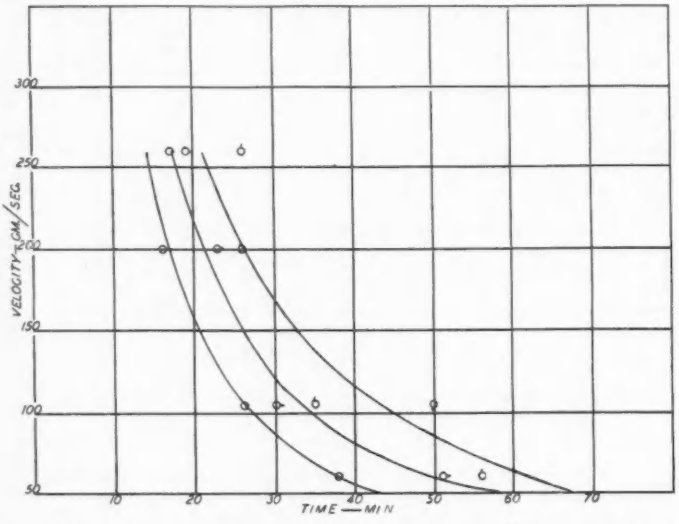

FIGURE 4. Change in time of drying to produce tacky surface. Dew-point constant at 17°C.

that the time to dryness is reached in 35 minutes, and the temperature of the fish muscle does not rise above 21°C. Under average conditions this is satisfied for single fillet treatment if the dry-bulb temperature is set 15°C. above the dew-point, and the wet-bulb temperature remains below 21°C. at an air velocity of 125-130 cm. per second.

EFFECTS OF OTHER VARIABLES UNDER STANDARD DRYING CONDITIONS

BRINING

Dreyer (unpub.), Dauphinee (unpub.), Forbes (1926), McFarlane (1933) and Lemon (1934) have investigated the effects of changes in brining conditions. In no case was drying standardized, therefore the subject was re-investigated, for the consequences of changes in drying conditions have been shown to be large.

EXPERIMENTAL

Three changes in brining conditions were recognized, strength of brine, temperature of brine, and time of immersion. The latter is fixed for each concentration by the flavour required. The effect of temperature (between 0° and 19°C.) on the penetration of salt into the fish was not found to be sufficient to appreciably influence the time of immersion to produce a required taste.

Fillets freshly cut from fish stored in ice for four days after killing were treated with brines of different strengths and at different temperatures for the definite length of time determined by taste as explained above. These were dried under the standard drying conditions given above. Investigations were made of the following sub-treatments:

(1) rinsing in fresh water after brining,

(2) rinsing in weak $NaHCO_3$,

(3) addition of bicarbonate to brine.

Brine concentrations used were limited to three, namely 60, 80, and 100 per cent saturated. These were chosen because when immersed in 60 per cent saturated brine, fillets gain weight, in 80 per cent their weight remains constant, and in 100 per cent they lose weight, as Beatty and Chipman (unpublished MS.) have shown.

Temperatures were limited to between 1° and 19°C. for obvious reasons.

The results are summarized in table II.

TABLE II. The effect on sheen and surface stability of varying concentrations and temperatures of the brine when drying conditions are constant.

Concentration of brine (per cent of saturation)	60		80			100		
Temperature of brine (°C.)	3	18	3	7	16	3	7	16
Sheen	4	2	4	3	2	2	2	2
Surface	2	1	4	3	2	4	3	2
Time to desired flavour (minutes)	20	20	15	15	15	8	8	8

DISCUSSION OF RESULTS

The results show that a low temperature is desirable, and that the most suitable concentration is 80 per cent saturated. The sheen formed on those fillets treated in 60 per cent saturated brine was better than that at any other concentration studied, but the difference was not sufficiently great to offset the longer period of drying required. Permanence of surface was increased by treatment in saturated solution but is offset by the fact that saturated brines cause severe wrinkling.

The various sub-treatments between brining and drying proved to be of no advantage. Slight improvement in sheen was observed when a fresh water rinse was used, but the surface stability was adversely affected.

The following brands of salt were compared under similar conditions. No

differences were noticed. Malagash Fisheries, Malagash Evaporated, Turk's Island, Torre Vieja, Trapani, Liverpool Evaporated, German Mined, and Cagliari.

The results given above were used to establish a standard method of brining as follows—treatment in 80 per cent saturated brine at 2-5°C. for 12 to 15 minutes.

Time of Storage at Ice Temperature

Commercial operators have supposed that the permanence of surface and of sheen are functions of the time of storage of the dead fish at ice temperatures. An investigation of these variables was undertaken.

The round fish were stored in ice for periods varying from 0 to 5 days. Freshly cut fillets were brined and dried by the standard methods.

The results are summarized in table III.

Table III. The effect on sheen and surface stability of variation in the time of preliminary storage in ice when brining and drying conditions are constant.

Storage time (days)	0	1	2	3	4
Sheen	0	3	1	3	4
Surface	0	3	1	3	3

The results partially substantiate the postulate of commercial operators, the exception being the minimum noticed for two-day fish. No explanation is offered for this. The results represent average values for large numbers of fillets and were repeated several times. The minimum was observed in each series.

GENERAL CONCLUSIONS

Age and condition of fish. Fillets should be cut from unspawned fish which have been stored in ice for periods longer than two days.

Brining. Fillets should be treated in 80 per cent saturated brine at a temperature between 1 and 7°C. for approximately 15 minutes.

Drying. Sets of conditions, within the following limits, which will dry the fish in 35 to 40 minutes and cause a 3 to 4 per cent weight loss are satisfactory.

Velocity. The limits are between 125 and 400 cm. per second, since below the former the fillets will not be at the wet-bulb temperature of the drying air, and above 400 they will be blown from their supports.

Wet-bulb temperature. Between 15°C. and 23°C., since below 15°C. there is danger of moisture condensation on the surface of the fillet in the smoke-house, and above 23°C. the fillet will be badly softened and partially cooked.

Humidity. It is clear from the results that humidities above approximately 70 per cent are unsuitable.

TECHNICAL EXPERIMENTS

The purpose of this part of the investigation was to obtain sufficient data for the design of efficient commercial driers. It was essential to carry out experi-

ments using semi-commercial equipment in order to check the experimental results obtained on the smaller scale, and to obtain the limiting conditions pertaining to tunnels.

A tunnel 6.09 m. long and 0.37 sq. m. cross-sectional area was used. The tunnel and control arrangements are shown in figure 5.

The tunnel was operated as a parallel current, adiabatic continuous drier. Air conditions were controlled in the usual manner, and air speeds measured by means of calibrated hot-wire anemometers.

The fillets were hung on tenters so placed on small cars that all long sides of the fish were parallel to the direction of air flow.

Experimental conditions common to all runs were as follows:

Air speed.	175 cm. per sec.
Brining.	15 min. in 80 per cent saturated salt at 7°C.
Draining.	The fillets were allowed to stand until they had reached the wet-bulb temperature of the air in the tunnel.
Loading.	32 kg. of fillets per cu. m.
Weight loss.	3 to 4 per cent.
Drying time.	35 to 40 minutes.

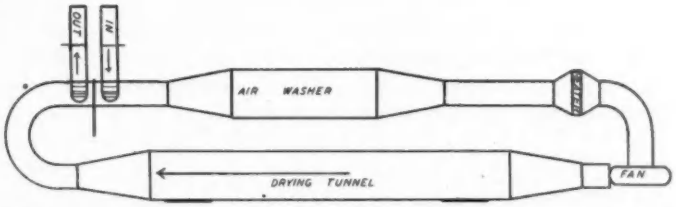

FIGURE 5. Sketch of semi-commercial tunnel and control arrangements.

RESULTS

Details of four experiments using the above arrangement follow.

Experiment no. 1. Preparation of 150 kg. of fillets.

Air conditions (entering).	Dry-bulb	40.5°C.
	Dew-point	21.0°C.
	Wet-bulb	26.1°C.
Average temperature of fish after treatment (centre)		26.1°C.
Weight loss		3 to 4 per cent.

Surface, unsatisfactory; sheen, very poor; muscle, soft, weak, and partially cooked.

Experiment no. 2. Preparation of 30 kg. of fillets.

Air conditions (entering).	Dry-bulb	33.3°C.
	Dew-point	16.1°C.
	Wet-bulb	21.1°C.
Average temperature of fish after treatment		21.1°C.

Surface and sheen, satisfactory; muscle, firm; fish suitable for smoking.

Experiment no. 3. Preparation of 100 kg. of fillets.

Air conditions (entering).	Dry-bulb	28.2°C.
	Dew-point	11.7°C.
	Wet-bulb	18.3°C.
Average temperature of fish after treatment		18.3°C.
Weight loss		3 to 4 per cent.

These fillets were completely satisfactory for commercial purposes, a representative of a commercial organization who followed a repetition of this experiment pronouncing them so.

Discussion

The results of these experiments corroborate the conclusions of the experimental part, namely, that the conditions in the tunnel must be such that the temperature of the fillets will remain below 23°C., that the mean outlet temperature of the fish at the centre is that of the wet-bulb temperature of the air, within experimental error, and that, if conditions be so adjusted within the limits described that a 3 to 4 per cent weight loss will occur in 35 to 40 minutes, a satisfactory surface and sheen will be obtained.

It is clear that outlet humidity conditions are a function of the tunnel air speed, other variables being constant. The limiting value for velocities greater than 250 cm. per second can be deduced from the experimental results. The limiting value is approximately 70 per cent relative.

Application

Conditions of loading, temperature of entering material, time of drying, and air speed being known, it is possible to make rough calculations for the limiting length of tunnel to be operated at a definite dew-point. The following examples will suffice:

Loading per cu.m.	Velocity (cm. per sec.)	Temperature of stock (°C.)	Drying time (minutes)	Dew-point (°C.)	Tunnel length (metres)
32	175	at wet-bulb	35	12.5	12.2
32	175	ditto	35	15.5	6.09
32	250	ditto	35	15.0	10.0

The limitations of such tunnels operated without dehumidification equipment can be judged from the following psychrometric data taken at Halifax, summer, 1935.

Dew-point (°C.)	Hour-days $\left(\frac{\text{hours}}{24}\right)$
17.2 and above	9.7
15.5 and above	17.9
12.5 and above	55.9

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Observations on Some Pacific Diatoms as the Food of Copepods and Fishes

By CHARLES W. LOWE
Department of Botany, University of Manitoba

(*Received for publication July 14, 1936*)

ABSTRACT

An investigation to determine what species of diatoms are used as food by different species of copepods: *Calanus tonsus*, *Euchaeta japonica*, *Metridia lucens*, *Harpacticus uniremis*, and *Diosaccus spinatus*. Also to determine the food chains between diatoms, copepods, and the following fish: Pacific herring, *Clupea pallasii*; chum salmon, *Oncorhynchus keta*; spring salmon, *O. tschawytscha*; dogfish, *Squalus suckleyi*; ling cod, *Ophiodon elongatus*; rockfish, *Sebastodes caurinus*; starry flounder, *Platichthys stellatus*; lemon sole, *Parophrys vetulus*; yellow-finned surffish, *Damalichthys vacca*; blue perch, *Taeniotoca lateralis*, and yellow shiner, *Cymatogaster aggregatus*. Stomach, intestines, and excreta were examined for the presence of diatoms. Almost all the common diatoms were found to be used as food by the copepods. The copepods and herring were found to be important links in the food chains between diatoms and the larger fishes.

This investigation has been carried out in order to determine which of the many species of diatoms occurring in the strait of Georgia are used as food by the various species of copepods and also to determine some of the food-chains between the diatoms and certain of the common fishes.

The material was secured partly by the author while at the Pacific Biological Station, Nanaimo, B.C., in the springs of 1930 and 1931, and partly from material supplied by that station subsequently.

There are about 150 species of diatoms known from around Vancouver island (Bailey and MacKay 1915, Bailey 1924, Gran and Angst 1931). Some of these belong to the littoral region and some to the open water. About 70 species are only occasional constituents of the plankton. A few species are plentiful all the year round, while others are abundant only at certain seasons.

The copepods particularly examined were *Calanus tonsus* Brady; *Euchaeta japonica* Marukawa; *Metridia lucens* Boeck; *Harpacticus uniremis* Kroyer; and *Diosaccus spinatus* Campbell. A pteropod (unidentified) which is very abundant in the plankton, was also used.

The following fishes were examined in order to determine the food-chains from diatom to fish:

(1) Pacific herring, *Clupea pallasii* Cuvier and Valenciennes;
(2) Chum salmon, *Oncorhynchus keta* (Walbaum), (young);
(3) Spring salmon, *Oncorhynchus tschawytscha* (Walbaum), (adult);
(4) Dogfish, *Squalus suckleyi* (Girard);

(5) Ling cod, *Ophiodon elongatus* Girard;
(6) Rockfish, *Sebastodes caurinus* (Richardson).
(7) Starry flounder, *Platichthys stellatus* (Pallas);
(8) Lemon sole, *Parophrys vetulus* Girard;
(9) Yellow-finned surffish, *Damalichthys vacca* Girard;
(10) Blue perch, *Taeniotoca lateralis* (Agassiz);
(11) Yellow shiner, *Cymatogaster aggregatus* Gibbons.

All material was obtained in, or near Departure bay, except three of the adult herring, one of which was taken off point Grey, and the other two near Pender harbour.

METHODS

Direct observations were made in the case of two shallow water copepods: *Harpacticus uniremis* and *Diosaccus spinatus*. These were kept in a watch-glass for a number of hours and supplied with the following species of diatoms: *Biddulphia aurita*, *B. roperiana*, *Melosira numuloides*, and *Licmophora lyngbyei*, all of which were eaten almost as fast as they could be supplied.

Qualitative determinations were carried out on the stomach contents of the copepods, pteropods and fishes.

Examinations were made of excreta and intestinal contents.

In order to determine the contents of the alimentary canals of the copepods it was found necessary to kill them as soon as possible after collection, otherwise the contents were soon expelled. A few individuals of each of the species mentioned were dissected under the low power of the microscope and contents determined in this manner. When dealing with large numbers of any species, the material was thoroughly washed to free it of any diatoms which might be adhering to it, and then boiled in strong nitric acid. This treatment removed almost all the organic matter and left the diatoms clean and free for identification. The separation of a large number of copepods of a number of species from a mixed plankton is a very long and tedious process, consequently only those large enough to be readily identified were separated from the mass of material collected. In this way, samples of *Calanus tonsus*, *Euchaeta japonica*, and *Metridia lucens* were secured, each sample containing many hundreds of individuals. The pteropods were obtained likewise. Following this the organisms of the plankton that were not copepods were removed leaving a mass of material made up almost entirely of the smaller copepods. This was the material used for the results in column six of the chart under the name "mixed small copepods".

In the larger fishes the stomach and the intestinal contents were cleaned and examined separately. This was done in spring salmon, ling cod, dogfish, rockfish and the flatfishes. In the spring salmon the small fish within the stomach were too much digested for dissection and the whole mass was cleaned with nitric acid.

The ling cod contained a few herring fit for dissection, but the copepods within the herring were too disintegrated for identification or dissection. The total contents of the ling cod stomach were cleaned altogether.

The dogfish stomachs contained a number of small herring in a fairly fresh state and when dissected revealed a number of copepods and other small crustaceans; diatoms were found in the copepods that were still in a condition for dissection.

From the rockfish stomachs four herring fit for dissection were obtained but only one revealed copepods which could be identified and from which diatoms were found.

There was no remarkable difference between the diatom content of the stomachs examined and the diatoms obtained from the intestines.

The excreta of the copepods were easily obtained from a number that were kept for a short time in shallow-glass dishes. The cylindrical pellets could be picked out with very fine forceps, or with a small pipette, and could be examined without cleaning. Excreta were obtained from two rockfish and from some surffishes which were kept in an aquarium for a few hours. This material was likewise treated with nitric acid.

RESULTS OF INVESTIGATION

COPEPODA

Two of the copepods used are species that are abundant in the water near land; these were *Harpacticus uniremis* and *Diosaccus spinatus*. From these were obtained largely diatoms that are abundant only near the shore. *Calanus tonsus*, *Euchaeta japonica*, *Metridia lucens*, and the mixed collection of small copepods, all of which were collected a few miles off shore, when examined contained largely diatom species characteristic of the open water. The only organisms other than diatoms obtained from these copepods were two forms of the silicoflagellate, *Distephanus speculum* Ehr. The small pteropods gave the same results as the open water copepods. Other smaller organisms may have been used as food by the copepods, but there was nothing to indicate their presence either in the stomach contents or excreta examined. Apparently the food of the copepods and the pteropods was almost entirely of diatoms.

HERRING (*CLUPEA PALLASII*)

15 specimens, about 2 months old, 4.1 to 4.4 cm. long.

The stomachs were found to be largely filled with copepods, pteropods and barnacle nauplii. Among the copepods the following were recognized: *Calanus tonsus*, Brady; *Paracalanus parvus* (Claus) Sars; *Acartia longiremis* (Lilljeborg) Sars; and *Pseudocalanus* (sp.?), and in a number of copepods diatoms were clearly visible. The diatom most common was *Thalassiosira decipiens*, but almost as abundant was *Skeletonema costatum*. A number of other species were present in much smaller quantity. In the stomach of one of these herring a quantity of *Thalassiosira decipiens* was found free and in a fresh state, showing clearly that this fish had eaten diatoms just prior to being caught.

4 specimens 18.2 to 23.0 cm. long.

The stomach of one of these was filled with *Euphausia* (sp?), while the other three contained a mixture of small crustaceans among which *Calanus tonsus* was most common. A few *Calanus* were fit for further examination and from

them a number of broken *Coscinodiscus* valves were observed including *C. excentricus* and *C. wailesii*. When all the stomach and intestinal contents of the herring were cleaned twenty-four species of diatoms were identified.

LIST OF DIATOMS AND THE ANIMALS IN WHICH THEY WERE FOUND

DIATOMS	ZOOPLANKTON							FISHES										
	Calanus tenuis	Eucharta japonica	Metridia lucens	Harpacticus uniremis	Diosaccus spinatus	Mixed small copepods	Pteropods	Young Herring	Adult Herring	Young Dog Salmon	Adult Spring Salmon	Ling Cod	Rock fish	Dog fish	Starry Flounder	Lemon Sole	Yellow Shiner	Yellow-finned Surf fish	Blue Perch
Acnanthes brevipes		X					X		X				X	X			X	X	X
" longipes					X		X			X			X	X					
Acnanthidium														X					
Actinocyclus ralfsii									X						X	X			
Actinoptychus splendens	X					X	X												
" undulatus						X	X								X	X			
Amphora marina	X								X				X		X				
" salina									X				X						
Arachnoidiscus ehrenbergii				X	X									X	X	X			
Asterionella japonica	X		X				X				X		X	X					
Asteromphalus hepactis														X		X			
Auliscus sculptus															X				
Bacteriastrum delicatulum		X	X			X								X					
Biddulphia arctica												X	X	X	X	X	X	X	X
" aurita	X	X		X	X						X	X	X		X	X	X		X
" roperiana				X	X						X			X	X	X	X	X	X
" vesiculosa						X	X					X	X						
Campylodiscus thuretii															X	X			
Coccomeis pellucida									X			X							
" scutellum	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Coscinodiscus angstii		X					X						X		X		X		
" centralis var. pacificus		X				X			X		X	X	X	X			X		X
" curvatulus	X	X	X			X	X				X	X		X	X	X	X	X	X
" marginatus	X	X				X	X	X											
" radiatus	X	X	X			X	X		X	X	X	X	X	X	X	X			
" subtilis	X	X				X	X	X							X				
" excentricus	X	X	X			X	X		X	X	X	X		X		X	X		
" wailesii	X	X	X			X	X	X	X	X	X		X	X	X				
Chaetoceros atlanticus	X													X					
" concavicornis	X	X	X						X						X		X		X
" convolutus	X												X						
" debilis	X																		
" didymus	X		X				X					X		X	X				
" laciniosus	X												X					X	
" radicans											X								
" vanheurckii																			
Corethron hystrix	X																		
Dactyliosolen mediterraneus		X					X												
Ditylum brightwellii	X																		
Fragillaria striatula		X							X					X					

CHUM SALMON (*ONCORHYNCHUS KETA*)

Specimen 3.9 cm. long.

Approximately 40 per cent of the stomach contents of this fish consisted of Harpacticidae, about 20 per cent of other copepods, and the remainder of schizopods, phyllopods, insect larvae and small flies. The copepods and phyllopods, when cleaned, yielded seventeen species of diatoms, of which *Biddulphia aurita*, *Cocconeis scutellum*, and *Licmophora lyngbyei* were the most frequent.

Spring Salmon (*Oncorhynchus tschawytscha*)

The alimentary canal of an adult spring salmon was supplied for the examination of the contents. The stomach was nearly filled with unrecognizable

LIST OF DIATOMS AND THE ANIMALS IN WHICH THEY WERE FOUND

DIATOMS	ZOOPLANKTON							FISHES										
	Calanus tonsus	Eubeta japonica	Metridia lucens	Harpacticus uniremis	Dioecesur sphinatus	Mixed small copepods	Pteropods	Young Herring	Adult Herring	Young Dog Salmon	Adult Spring Salmon	Ling Cod	Rock fish	Dog fish	Starry Flounder	Lemon Sole	Yellow Shiner	Yellow-finned Surf fish	Blue Perch
Grammatophora angulosa var. hamulifera	X								X		X								
" marina	X			X	X	X	X					X	X		X	X	X	X	X
" serpentina	X					X	X						X		X		X	X	X
Cyrocigma hippocampus													X		X				
Isthmia nervosa				X	X				X		X		X	X	X		X	X	X
Licmophora lyngbyei	X	X		X	X		X	X	X	X		X	X	X	X	X	X	X	X
" paradoxa				X	X			X					X	X	X	X			
Melosira juergensii	X	X	X	X	X	X	X	X	X		X			X	X	X			
" moniliformis	X			X	X				X	X				X	X	X	X		X
" nummuloides	X	X		X	X				X					X	X		X		
Navicula ambigua	X						X												
" crabro		X				X	X					X	X		X	X			
" " var. explota												X							
" didyma	X														X		X		
" directa										X					X				
" distans	X						X												
" liber															X				
" lyra	X								X	X		X			X		X	X	X
" smithii	X								X				X						
Nitzschia paradoxa	X										X				X	X			
" seriata	X	X	X			X	X	X		X		X	X	X	X				
" sigma	X																		
" closterium									X										
Parelia sulcata	X		X	X		X	X				X	X	X		X	X			
Plagiogramma gregorianum	X																		
Pleurosigma angulatum	X	X					X			X	X		X	X	X	X	X		
" formosum		X								X			X		X				
" rigidum		X												X	X				
Rhabdonema arcuatum	X	X		X	X		X				X	X			X		X	X	X
Rhoicosphaenia curvata var. marina	X												X				X		
Skeletonema costatum	X	X						X	X			X	X	X	X	X	X	X	X
Stephanopyxis palmeriana	X											X							
Synedra affinis											X								
Thalassiosira aestivalis	X							X											
" decipiens		X						X						X					
" nordenskioeldii	X																		
" pacifica	X	X				X	X		X		X	X	X	X	X		X	X	
" rotula	X																		
Thalassiothrix nitzschioides		X				X	X		X	X							X		
" longissima	X	X				X	X					X	X	X					
Trachyneis aspera var. intermedia										X									
Tropidoneis antarctica var. polyplasta															X		X		X

remains of small fishes, probably herring, which, after treatment, showed the presence of eleven species of diatoms. The material removed from the intestine revealed the same species with eight additional, making a total of nineteen from this one fish. It was found in this case that diatom remains were plentiful but more fragmentary than in the other fishes examined; only a few perfect diatom

valves were observed. *Coscinodiscus wailesii* and *C. excentricus* were more numerous than the other species found here.

LING COD (*OPHIODON ELONGATUS*)

1 specimen 72.5 cm. in length.

Its stomach was crowded with young herring. A few of the herring that were fit for dissection showed that their stomachs contained copepods in excess of other food. Twenty species of diatoms were obtained altogether from the stomach of this fish and from the intestines likewise.

ROCKFISH (*SEBASTODES CAURINUS*)

Specimens from 25 to 31 cm. long were examined.

The stomachs of two of them contained the remains of small crabs and fish that were probably young herring; the other three contained only small fishes that, as far as could be judged, were all herring. Only four of the herring were in a condition fit for dissection; one contained a number of *Diosaccus spinatus*, two were filled with remains of small crustaceans, and the fourth was empty. The stomach contents of all the rockfish when cleaned showed 20 species of diatoms and the excreta and intestinal contents revealed 20 species, 32 species being obtained altogether.

DOGFISH (*SQUALUS SUCKLEYI*)

The three dogfish examined, were netted while they were following a school of small herring and the stomach of each dogfish was filled with these young fish. Many of the herring showed that they had been feeding upon copepods amongst which *Diosaccus* and *Harpacticus* were common. The best conditioned copepods were found to contain diatoms, chiefly broken remains of *Biddulphia aurita*, *B. roperiana*, *Coscinodiscus* spp., and *Melosira* spp. Thirty species of diatoms were obtained when the stomach and intestine contents were cleaned.

STARRY FLOUNDERS (*PLATICHTHYS STELLATUS*)

12 specimens, 2 only 4 cm., the others from 25 to 38 cm. in length examined.

The contents of the alimentary canals of the small ones when cleaned showed the following diatoms of the littoral zone: *Biddulphia roperiana*, *Grammatophora marina*, *G. serpentina*, *Rhabdonema arcuatum*, *Licmophora lyngbyei*, and *Actinocyclus ralfsii*. The larger flounders were found to be filled chiefly with a mixture of small molluscs and in two there was also a quantity of the green alga, *Ulva*. This material, along with the contents of the intestines, when cleaned revealed 42 species of diatoms. *Bidulphia roperiana* was the most common in both the small and the large fish. The other species which occurred frequently were *B. aurita*, *Rhabdonema arcuatum*, *Grammatophora marina*, *G. serpentina*, and *Trachyneis aspera* v. *intermedia*. None of the true plankton species was frequent.

LEMON SOLES (*PAROPHRYS VETULUS*)

5 specimens, 18 to 26 cm.

As in the flounders, the stomach contents were chiefly small molluscs. The total contents of the alimentary canal when cleaned revealed 23 species of diatoms that were largely sedentary shore forms.

SURFFISHES (*EMBIOTOCIDAE*)

Yellow shiner (*Cymatogaster aggregatus*), 10 specimens, 15.5 to 17 cm. Yellow-finned surffish (*Damalichthys vacca*), 7 specimens, 11 to 13.5 cm. Blue perch (*Taeniotoca lateralis*), 8 specimens, 17 to 19 cm. long. The stomach contents in all of them were almost entirely made up of the smaller crustaceans, including a number of Harpacticidae. From the yellow shiner, 25 species of diatoms were obtained, from the yellow-finned surffish, 13, and from the blue perch, 16. The majority of species were largely those of the littoral zone.

DISCUSSION AND CONCLUSIONS

The lists (pages 15 and 16) show the species of diatoms which were found in the digestive tracts of the various plankton animals and fishes examined. There are 82 species and varieties recorded which is slightly more than half the species known to occur in the waters around Vancouver island throughout the year.

The fact that some species were found in most of the animals studied does not necessarily mean that they are abundant in the plankton or elsewhere. It may be that the valves are not so easily broken as in the more delicate species, or if broken, the fragments show some character by which identification can be made. Examples of such diatoms are *Archnoidiscus ehrenbergii*, *Cocconeis scutellum*, *Gramatophora* sp. and some species of *Coscinodiscus*. Other species of diatoms, which are always more or less common at the time most of the material was collected, were never observed from the stomach contents. The different species of *Rhizosolenia*, others like *Stephanopyxis nipponica*, and most species of *Chaetoceros*, are so formed that upon ingestion by any small animal they are broken into fragments and only occasionally can they be identified.

A study of the chart shows that almost all the common diatoms are used as food by the copepods. Those that are found in shallow water or near the shore feed almost entirely upon diatoms of the littoral zone. *Harpacticus uniremis* and *Diosaccus spinatus* were found to contain largely species of *Acnanthes*, *Arachnoidiscus*, *Biddulphia*, *Grammatophora*, *Licmorphora*, *Paralia*, and others which are either sedentary or littoral. On the other hand the deep water copepods like *Euchaeta japonica*, *Calanus tonsus*, *Metridia lucens*, were found to feed upon the true plankton diatoms such as *Asterionella japonica*, *Bacteriastrum delicatulum*, *Chaetoceros* sp. and many others.

The greatest number of diatom species found within the fishes or copepods examined was in *Calanus tonsus* from which 46 species were identified.

The food-chains for the fishes examined here show the importance of the

young herring to the larger fishes and of the diatoms to all the fishes. The food-chains may be charted as below:

Fish	Food, 1st degree	Food, 2nd degree	Food, nth degree
Spring salmon	herring	copepods	diatoms
Ling cod	"	"	"
Rockfish	"	"	"
Dogfish	"	"	"
Adult herring	crustaceans		"
Flounder	molluscs		"
Lemon sole	"		"

The observations made here upon the herring compare favourably with those made by Lebour (1918, 1925) at Plymouth, England, upon the herring *Clupea harengus* L. and the sprat *Clupea sprattus* L. These fish were all smaller and younger than those examined by the present author from the Pacific coast. Lebour clearly demonstrated that the young herring and the young sprat, in the post-larval stage, fed upon diatoms, larval molluscs, and small copepods and further that the young molluscs and copepods fed upon diatoms and the flagellated green alga, *Halosphaera viridis*. This alga was not present in the Pacific coast plankton at the time the herring from these waters were captured. Wailes and Hart (1932) and Wailes (1935) have recorded the occurrence of many species of diatoms in the digestive tracts of pilchards and herring in southern British Columbia waters. Mann (1921) examined the stomach contents of some hake and found them gorged with small herring; the herring in turn were filled with copepods and the copepods with diatoms. He concluded his observations with the remark, "No diatoms, no hake".

The present investigation serves to give some indication of the importance of diatoms in the food cycle in the coastal waters of British Columbia.

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Food of the Coarse-Scaled Sucker (*Catostomus macrocheilus* Girard)

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ABSTRACT

Examination of 167 specimens shows that immature fish feed upon planktonic forms while mature fish feed upon larger bottom organisms and bottom detritus. The diet varies with habitat and season, depending upon what is available. Since it eats the spawn of salmonids and takes much the same food organisms as they do, its presence in waters containing important salmonids is undesirable.

INTRODUCTION

The problem of the relation of so-called coarse fish to the production of game and commercial fish is one which calls for a great deal of careful investigation. A fundamental phase is the study of the foods and food-chains of all the species involved and the present report on the food of the coarse-scaled sucker, *Catostomus macrocheilus* Girard, is a contribution in this regard. As far as the writer is aware, no data are available on the food of this fish. For the eastern common or white sucker, *Catostomus commersonii* (Lacépède), a considerable amount of information is available as a result of the publications of Forbes (1890), Reighard (1915), Pearse (1918), Clemens and associates (1923), Bigelow (1923), Stewart (1926) and others.

MATERIAL

The material examined consists of the digestive tracts of 167 individuals, and the record of 31 specimens of the fine-scaled sucker, *Catostomus catostomus* (Forster), from Eagle river is given for comparison. The suckers were collected by Dr. D. McCaffrey from Blue lake, Princeton, in 1933 and 1934, by Mr. W. F. Baxter in Eagle river at Malakwa in 1932 and 1933, by Dominion fishery officers in various parts of the province and by Dr. W. A. Clemens in Shuswap lake and Okanagan lake (as well as Woods and Duck lakes). The data in regard to the latter fish (48 specimens) will be used in the report on the Okanagan survey but are presented here also, in order to give a more complete picture. Dr. C. McC. Mottley kindly supplied the data on the food of the Kamloops trout, *Salmo gairdneri kamloops* Jordan. The writer is greatly indebted to all these persons. The study was carried out in part in the Department of Zoology of the University of British Columbia and in part at the Pacific Biological Station and the facilities provided are much appreciated.

METHODS

Since the alimentary tracts contained numerous small organisms mixed with considerable quantities of sand and bottom ooze, it was impracticable to attempt to measure the food material or the actual volume of the individual constituents. Hence the investigation was confined to the identification of the organisms occurring, to actual counts of individuals in the case of young fish and to a record of the number of tracts containing each food item. In addition there was recorded the presence of bottom ooze (detritus) and sand, as indicating bottom feeding.

DISCUSSION OF RESULTS

The results of the examinations are given in tables I, II, III and IV.

TABLE I. List of food organisms and other materials found in the digestive tracts of the coarse-scaled sucker, *Catostomus macrocheilus*

	PLANTS
Filamentous algae:	*Nostoc, Spirogyra, Mougeotia, Ulothrix, Chara.*
Desmidiaceae:	*Closterium.*
Diatomaceae:	*Synedra, Navicula, Fragillaria, Surirella, Tabellaria, Melosira.*
Bryophyta:	*Selaginella, Sphagnum.*
	ANIMALS
Protozoa:	*Difflugia.*
Annelida:	*Lumbriculus.*
Bryozoa:	*Plumatella polymorpha.*
Ostracoda:	Two unidentified species.
Copepoda:	*Epischura nevadensis, Diaptomus ashlandi, Cyclops bicuspidatus, Cyclops viridis, Canthocamptus minutus.*
Cladocera:	*Daphnia longispina, Acroperus harpae, Scapholeberis mucronata, Pleuroxus denticulatus, Pleuroxus* sp., *Bosmina longispina, Eurycercus lamellatus, Chydorus sphaericus, Alona costata, Alona affinis, Leptodora kindtii, Leydigia quadrangularis.*
Amphipoda:	*Gammarus limnaeus, Hyalella azteci.*
Hydracarina:	Two unidentified species.
Insecta:	Plecoptera nymphs, Ephemerida nymphs, Odonata (including *Ischnura* sp. nymphs), Hemiptera (*Corixa* sp.), Trichoptera (*Setodes* sp., *Hydropsyche* sp., and other larvae), Simuliidae larvae, Culicidae (*Chaoborus* sp. larvae), Chironomidae larvae, Tipulidae larvae.
Mollusca:	*Planorbis* sp., *Gyraulus vermicularis, Valvata lewisi, Cochliopa* sp., *Musculium truncatum.*
	MISCELLANEOUS
	Insect eggs, fish eggs (*Oncorhynchus nerka kennerlyi*), wood fibres, seed capsules, conifer needles, organic detritus, sand, gravel.

TABLE II. Comparison of diet of immature and mature *C. macrocheilus*, based upon percentage of occurrences

	Immature fish		Mature fish	
	Shuswap lake 42 stomachs	Okanagan lakes 22 stomachs	Blue, Fraser and Tabor lakes 43 stomachs	Okanagan lakes 20 stomachs
	%	%	%	%
Ostracoda	55	4	11	30
Copepoda	67	17	3	55
Cladocera	98	38	5	45
Amphipoda	0	0	12	5
Hydracarina	50	4	7	15
Aquatic insect larvae	94	85	80	85
Terrestrial insects	7	0	7	10
Mollusca	0	0	20	10
Plants, diatoms, algae	10	17	16	40
Detritus	72	61	85	80

TABLE III. Percentages of occurrences of various food items in the diet of *C. macrocheilus* from different localities and at different seasons, and also of *C. catostomus*.

	Lake fish			Stream fish	
	C. macrocheilus			*C. macrocheilus*	*C. catostomus*
	June-July Blue lake 32 fish	July-Aug. Okanagan 20 fish	Nov.-Dec. Cluculz 12 fish	Oct.-Nov. Eagle R. 20 fish	Oct.-Nov. Eagle R. 31 fish
	%	%	%	%	%
Ostracoda	19	35	0	0	13
Copepoda	0	60	8	0	6
Amphipoda	16	5	50	0	0
Trichoptera	50	45	33	15	19
Chironomidae	56	85	100	50	54
Other aquatic larvae	40	15	25	85	29
Mollusca	37	10	67	0	0
Fish eggs	0	0	58	20	0
Diatoms	22	10	0	25	10
Algae	12	5	25	85	42
Sand	0	55	33	15	42
Detritus	87	85	75	35	35
No food	0	0	0	20	19

TABLE IV. Comparison of diet of adult suckers (*C. macrocheilus*) and Kamloops trout (*Salmo gairdneri kamloops*)

	Percentage occurrence in 241 stomachs of Kamloops trout (Paul lake)	Percentage occurrence in 87 stomachs of coarse-scaled sucker (Blue, Cluculz and Okanagan lakes)
Hirudinea	5	0
Ostracoda	0	15
Copepoda	3	15
Cladocera	5	16
Amphipoda	55	14
Hydracarina	0.5	10
Aquatic insects	48	78
Terrestrial insects	15	4
Mollusca	10	25
Plants: diatoms, algae	6	38
Detritus	0	72

An examination of these results indicates that the food of the coarse-scaled sucker consists almost entirely of bottom forms and of organisms associated with bottom vegetation.

In the case of the immature fish the greater part of the food is made up of plankton organisms and aquatic insect larvae mixed with small quantities of bottom ooze. The planktonic forms include Cladocera, Copepoda, Hydracarina and Ostracoda, while the insect larvae are those of Chironomidae, Trichoptera and Ephemerida. The habitat of all of these organisms is on or near the bottom.

Stewart (1926) points out that in the development of the eastern sucker five periods may be distinguished on the basis of its feeding activities, namely, yolk-food period, top-feeding period, critical period, fingerling period and adult period. Some of these can be correlated with the change of the mouth from a terminal to an inferior position. This investigator presents data to show that after the yolk-sac is absorbed, the young feed on planktonic forms near the surface, then as the mouth descends the fish include organisms associated with the bottom until, in the fingerling stage, the diet is made up almost entirely of bottom animals and plants. The examination of the material from the immature Shuswap lake suckers shows that these fish are comparable to those of the fingerling stage as recognized by Stewart, inasmuch as the food of both species at this stage appears to be very similar.

The food of the mature coarse-scaled sucker resembles that of the young; the same types of organisms are found in the digestive tracts of both but in different relative amounts. Large quantities of bottom ooze and associated organisms such as Chironomidae larvae, Mollusca (snails, small clams) and Amphipoda are taken, while planktonic animals occur only in a few cases and in small numbers. The occurrence of the larger organisms as items of diet is no doubt related to the larger size of the fish. The quantities of bottom ooze in most of the specimens examined shows that the mature sucker feeds directly

off the bottom, and the character of the food materials indicates that it does not select its food.

The change in diet as between young and mature fish is shown in table II, which includes data from suckers from Shuswap, Blue and Tabor lakes, the Fraser river and some Okanagan lakes. In the case of the latter, the results do not show such a pronounced change as do those from the other localities because of the smaller size differences between the two groups of fish.

Variation of Diet with Habitat and Season

Since the sucker material was gathered from several different localities and at different seasons of the year, the contents of the alimentary tracts show corresponding differences. These variations are shown in table III, which also includes material from 31 specimens of the fine-scaled sucker for comparative purposes. The diet of the fish collected from the various lakes is very similar except for differences resulting from the seasonal variations of organisms. Seasonal forms such as ostracods, copepods and aquatic insect larvae are found more often in the fish collected during the summer months than in those taken later in the year. The diet of the lake fish differs considerably, however, from that of fish taken from streams. Stream fish appear to feed upon more algae, diatoms and aquatic insect larvae other than Chironomidae, while lake fish include Amphipoda and Mollusca. The differences are evidently not due to choice but to circumstance, since the organisms devoured by the fish are those which are peculiar to the habitat. This conclusion is substantiated by the fact that there is very little difference shown between the diets of the two species of suckers collected from Eagle river.

The Sucker as a Food Competitor

The food of the coarse-scaled sucker consists of organisms which also constitute part of the diet of commercial and game fishes. Foerster (1925) found that the food of yearling sockeye salmon, when migrating from lakes, consisted of chironomid and other insect larvae together with plankton organisms such as Copepoda and Cladocera. A similar diet has been shown for the Kamloops trout by Mottley and Mottley (1932). In table IV are presented data showing the overlapping of food materials taken by mature suckers and Kamloops trout, particularly evident in the cases of Amphipoda, Mollusca and aquatic insect larvae.

The Sucker as a Spawn Eater

Besides the competition for food which appears to exist between suckers and commercial and game fishes, there is also the spawn-eating habit of the former to be considered. Stewart (1926) concludes from his investigation that fish eggs are rarely taken by the Eastern species. However, Atkinson (1931) reports the destruction of the eggs of the grey trout, *Cristivomer namaycush* (Walbaum), by this species. The present data show that the coarse-scaled sucker feeds upon fish eggs on occasion. Over half of the specimens from Cluculz

lake contained one or more fish eggs and several fish collected in Eagle river were gorged with eggs, the gut of one containing 125 undigested as well as the remains of many more. The eggs in the latter case were probably those of the kokanee, *Oncorhynchus nerka kennerlyi*, since these fish were spawning at the time of collection (October and November). It is interesting to note that the fine-scaled suckers, which were collected at the same time and place, showed no evidence of having fed upon salmon eggs.

CONCLUSION

The common sucker of British Columbia does not appear to possess any qualities making it a desirable species. Analyses of the intestinal contents of both fingerlings and adults show that the sucker subsists upon food organisms which also constitute part of the diet of the more valuable fishes. It should therefore be recognized as a serious competitor with them for food. The analyses have also shown that the adult sucker feeds upon the eggs of some of these fishes to such an extent that it should also be considered as a predator. Consideration of these facts shows that the presence of this coarse fish in waters containing commercial and game fish is undesirable.

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The Return from the Sea of Sockeye Salmon (*Oncorhynchus nerka*) with Special Reference to Percentage Survival, Sex Proportions and Progress of Migration

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ABSTRACT

From the marking of 104,061 seaward-migrating sockeye at Cultus lake, B.C., in 1930 and 365,265 in 1931, recoveries of 3,821 and 12,803 adults, respectively, were obtained, or 3.67 per cent and 3.5 per cent. Consideration of errors of 10 per cent and 5 per cent respectively, for marked adults missed and a differential mortality among marked fish ranging from 58 per cent to 65 per cent, probable value—62 per cent, indicate a normal survival in the ocean of between 3.5 per cent and 11.7 per cent, probable value, 9.9 per cent. Of eggs deposited naturally a mortality of 97.5 per cent to the seaward migrant stage occurs. The total loss during the life cycle thus amounts to approximately 99.75 per cent. On the basis of 4,500 eggs per sockeye female the ratio of spawning parents to returning mature offspring ranges from 1:2 to 1:6.5, most probable ratio —1:5.5. Females predominate in trap and seine catches and males in gill nets but the paucity of males in the spawning escapement is not clearly explained. Marked sockeye recoveries in the fishing areas indicate no regular geographic progression in the run except at the estuary of the Fraser river. Catches were made in all areas over the same periods but a lag at the river mouth was noted. Migration to the Fraser occurred through both Juan de Fuca and Johnstone straits. A particular spawning population does not necessarily, therefore, travel in a compact school nor follow the same route.

The present paper reports upon two successive experiments conducted at Cultus lake, British Columbia, to determine primarily the percentage return of adult sockeye salmon from the sea, as derived from a known number of seaward-migrating young. Data pertaining to sex ratios among adults and the progress of the migration through the fishing areas to Cultus lake are also assembled.

The importance of being able to compute the amount of natural mortality among sockeye salmon during their life history or, conversely, the percentage of survival which prevails, is obvious. To estimate the harvest, one must be able to compute the probable yield. To achieve this end other workers (Gilbert and Rich 1927; Holmes 1933), dealing with Alaska red-salmon, compared counts of adults returning from the sea with the numbers of spawners in the preceding cycle's spawning escapement. In such instances all losses, those occurring in both the fresh and salt water stages of the life history, were combined.

Any means whereby the mortality can be allocated to definite phases of the life-cycle are distinctly advantageous. As the former of the above-mentioned authorities (p. 64) remark, "we have no doubt, however, that if we could sub-

stitute for the number of spawning fish the number of resulting fry emerging from the gravels, or of still higher value, the number of fingerlings of the brood that accomplish their seaward migration, we would be in possession of data of far greater predictive value." Holmes (1933) also pointed out the importance of knowing what part of the mortality takes place in fresh water and what part occurs after the fingerlings reach the ocean. He attempted a division of the losses by marking a number of the seaward-migrating young, since a total count was impracticable, but the results were neither clear-cut nor conclusive.

In the present experiments not only was a complete count of all seaward-migrating sockeye available but the fresh-water mortality was known. In the course of an extended investigation of the propagation of sockeye salmon at Cultus lake (Foerster, 1929a, 1936b) counts of spawning adults, calculations of egg depositions or fry liberations and counts of the resulting down-stream migrants were obtained for a period of years. These data indicated the extent of loss during the year's residence in fresh-water and also the numbers of young sockeye migrating to the sea.

The mortality occurring subsequently in the ocean, however, was less readily computed. In the Alaskan studies referred to above, an enumeration of all returning adult salmon, whether caught in the commercial fishing areas or counted migrating up-river, could be obtained since the spawning run to each river system was deemed to be distinct and separable from all others. The sockeye returning to Cultus lake, however, formed a portion only of the Fraser river commercial catch and could not normally be segregated. It became necessary, therefore, to mark the Cultus lake migrants, by removing certain fins, in order that upon capture they might be readily identified.

METHODS

Counting Seaward Migrants

As intimated above, a counting fence, described in a previous paper (Foerster 1929c), had been in operation in Sweltzer creek, the outlet stream from Cultus lake, since 1926 for the enumeration of seaward-migrating young sockeye. A technique had been developed whereby every migrant arriving at the structure was counted and released. The seaward-migrants utilized for the present experiments were those contained in the migrations of 1930 and 1931 because it was known that they would not be too numerous to be readily handled.

Marking

The marking of salmon by removing certain fins has become an accepted method of providing a distinguishing characteristic whereby the adults may be readily identified. It has been used for a number of years with evident success, as recorded by Rich and Holmes (1929), and for sockeye salmon in paricular, at Cultus lake by Foerster (1934, 1936a) and in Alaska by Holmes (19t33).

Though the occurrence of migrants with fins naturally lacking had never been observed at Cultus lake nor were such found in the migrations of 1930 and 1931, to avoid any likelihood of confusion in identifying the returning marked adults, the possibility of which is outlined by Rich and Holmes (1929), two or

more fins were removed. In 1930 the mark consisted of the removal of the two pelvic fins only, while in 1931 the two pelvics and the adipose fin were clipped off, the presence or absence of the latter fin providing the distinction between the two years' migrations.

Since the down-stream migrants arrived at the counting fence during the night time they were held until the following day and then counted, marked and liberated. They were returned to the creek below the counting traps and allowed to continue upon their seaward journey. A close check of the creek

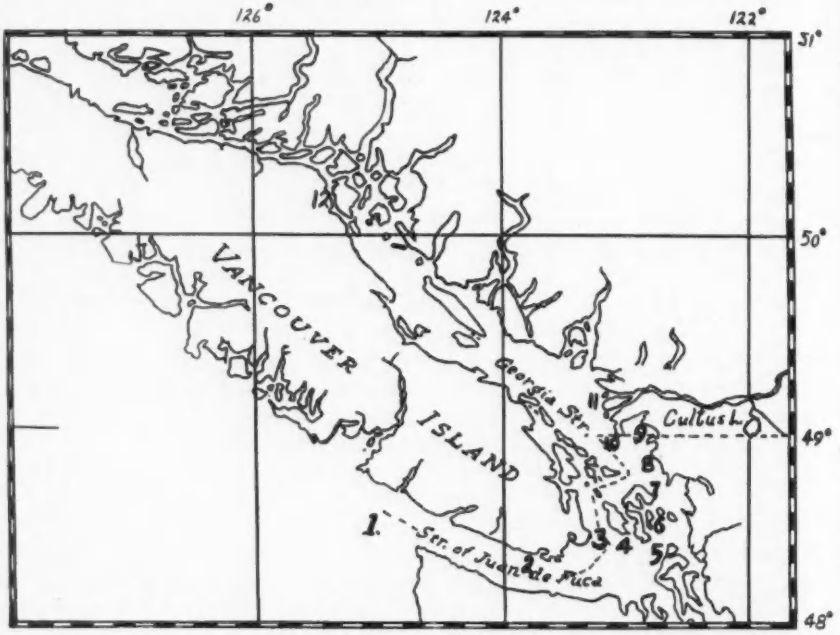

FIGURE 1. Map of southwestern British Columbia and northwestern Washington showing the principal fishing grounds traversed by sockeye salmon migrating to the Fraser river. The areas indicated by the numerals are: 1, Swiftsure banks; 2, Sooke traps; 3, Salmon banks; 4, Iceberg point; 5, local banks off Whidby I.; 6, Rosario strait; 7, Lummi island; 8, Birch bay; 9, Boundary bay; 10, point Roberts; 11, Fraser river and estuary; 12, Johnstone strait, Bear river and Deepwater bay.

below the point of liberation was made to ascertain what degree of mortality followed immediately on the marking operations. The losses were so few as to be negligible, estimated at less than 1 per 10,000.

RECOVERY OF RETURNING ADULTS

This phase of the experiments presented the greatest difficulty chiefly because of the wide-spread fishing grounds, extending, as shown in figure 1, from the Swiftsure banks in the open Pacific, through the strait of Juan de Fuca,

the San Juan archipelago and the strait of Georgia to the Fraser river. In 1932 the Washington State Fisheries Department generously co-operated by providing an observer in each of the canning centres operating in the state that season, namely, Friday Harbour, Bellingham and Everett, while the Biological Board of Canada stationed an assistant at the Empire cannery, Esquimalt, B.C. The duties of such officers were to scan all sockeyes entering the canneries, segregate the marked ones, and from the latter obtain length, weight and sex data and scale samples. From the Fraser river and the adjacent waters of the strait of Georgia, however, eight canneries were packing sockeye salmon and funds were not available to provide an observer at each. Accordingly, placards,

25C REWARD For Marked Sockeye
No. 1 - CULTUS LAKE. In fourth year.
No. 2 - CULTUS LAKE. In fifth year.
No. 3 - EAGLE RIVER. In fourth year.
No. 4 - EAGLE RIVER. In third or fifth year.
Fraser River SOCKEYE SALMON have been MARKED in four different ways, as indicated in the diagrams above, by CUTTING OFF certain FINS. THE FINS CUT OFF ARE SHOWN IN BLACK. Some of these Sockeye should return this season 1933.
TWENTY-FIVE CENTS 25c Will be Paid to Any Person Who Finds One of These Marked Sockeye, Sends in the Pieces of Skin Showing the Scars Where the Fins Were Cut Off and Gives the Following Information:
1. Date and Place of Capture.
2. Length in Inches and Weight in Pounds.
3. Sex of Fish. (M - for Male) (F - for Female.)
4. About 20 Scales. Clean off knife, scrape first from head to to tail to remove slime, then scrape from tail to head on side of fish between the dorsal fin and the lateral line, place scales on piece of paper which should then be folded.
R. E. FOERSTER PACIFIC BIOLOGICAL STATION NANAIMO, B. C.

FIGURE 2. Type of placard posted throughout the Fraser river area in 1933 to announce the marking of sockeye salmon and the payment of a reward for the sending in of scars, data and scales from marked individuals recovered.

similar to that exhibited in figure 2, were circulated throughout the area, fishermen and cannery employees were urged to co-operate and an effort made to obtain, by this means, a record of the marked fish observed. An officer visited each cannery regularly to collect the authentic scars and records and make payment of the rewards.

In 1933, the same methods were utilized, but the Biological Board financed the entire undertaking. In that year the number of canneries was greater, consisting of Esquimalt, Friday Harbor, Everett (2), Bellingham (3), Anacortes (5), Blaine and Fraser river (9). In addition the catches of sockeye at Quathiaski cove were brought into the program by means of paying rewards for recoveries submitted. During the peak of the season two observers were employed at both Anacortes and Bellingham, covering all the operating canneries at these points.

In 1934, when only five-year-old marked sockeye were expected to return, observers were located only at certain strategic canneries where the main catches from all districts would be sampled, namely, Everett, Bellingham, Esquimalt and the Imperial cannery on the Fraser river. The numbers of marked individuals recovered were accepted as an index of the probable total occurrence of marked sockeye throughout the fishing areas and the latter calculated on the basis of total landings.

The returns to Cultus lake were obtained when the annual counts of spawning sockeye, as elsewhere described (Foerster 1929a), were made. All sockeye reaching the counting weir below the lake were examined and an accurate record of marked individuals thus secured.

From all marked sockeye in the fishing areas the scars of the removed fins and scale samples were taken. The latter were mounted and examined and the authenticity of the mark, as far as age of the fish was concerned, established. In the great majority of cases the scars were clean and unquestionably authentic but occasionally partial regeneration of one or both pelvic fins occurred. Where only one pelvic fin scar was submitted, either with or without the adipose scar, the record was rejected unless the circumstantial evidence clearly indicated the recovery to be genuine. Such submissions were rare. In a few instances sockeye were recovered, lacking the pelvic fins, on which no scar appeared, the whole area being completely scaled over. Subsequent examination generally disclosed the absence of pelvic bones and, in agreement with Rich and Holmes (1929, pp. 218-219), these records were discarded as being caused by abnormal non-development of the pelvic girdle.

Compilation of Sockeye Landings

In compiling the data pertaining to the progress of marked Cultus lake sockeye salmon through the fishing areas, the records were tabulated according to place of capture and thereafter grouped into the twelve major areas recognized respectively by the Washington State Fisheries Department and the Dominion Department of Fisheries, and designated in figure 1. The catch records from all operating canneries were assembled and treated in a similar manner to show the seasonal progress of the sockeye fishery as a whole. While not considered as entirely accurate, due to the probable omission of unavailable records or the inability to segregate exactly into areas of capture, the pack returns are generally reliable and portray the trends in each of the fishing seasons.

THE RETURN FROM A KNOWN ESCAPEMENT

Sockeye salmon normally migrate seaward from Cultus lake at the beginning of their second year, but a proportion, varying from year to year, may pass to sea in their third, having remained in the lake a second season. The migrants marked in the migrations of both 1930 and 1931 may thus have been of both age groups. Sockeye normally return after two and a half years in the sea or in their fourth (4_2) and fifth (5_3) years respectively. Precocious three year (3_2)

and retarded five year (5_2) individuals frequently occur, having spent one year less and one year more, respectively, in the ocean than the normal group. Hence from each of the two migrations there may return the following age classes:

Year of seaward migration	Year of return as adults			
	1931	1932	1933	1934
1930	3_2	4_2+5_3	5_2	
1931		3_2	4_2+5_3	5_2

Therefore, while the majority of the recoveries were expected in 1932 for the first experiment and 1933 for the second, returns in the year previous and the year following, in each case, had to be anticipated.

Recoveries Obtained

The recoveries obtained for each of the two experiments have been set down in table I, divided as to year, location of capture and sex. For the moment, however, the totals are of chief concern and are found to signify *a return from the sea of 3.67 and 3.5 per cent, respectively, of marked down-stream migrants.*

Table I. Data for each of the two experiments, showing the numbers of migrants marked and the numbers of recoveries divided as to sex, place of recovery and year

	First experiment 1930-1933						Second experiment 1931-1934					
Number of migrants marked	104,061						365,265					
Recoveries obtained from canneries at:	1931 ♂	1931 ♀	1932 ♂	1932 ♀	1933 ♂	1933 ♀	1932 ♂	1932 ♀	1933 ♂	1933 ♀	1934 ♂	1934 ♀
Esquimalt			80	116	2	1	2	0	372	277	12	9
Friday Harbor			107	237					410	493		
Everett			73	95	2	0	6	0	854	1,002		
Anacortes					3	0			646	967	147	90
Bellingham			176	247	4	1	4	0	858	1,172		
Blaine									75	136		
Fraser river			480	339	12	11	1	0	1,038	772	125	15
Quathiaski									44	45		
Total for fishing areas			916	1,034	23	13	13	0	4,297	4,864	284	114
Recoveries at Cultus lake	18	10	465	1,334	3	5	28	0	1,022	1,834	127	149
Total recoveries	18	10	1,381 or 3,821	2,368	26	18	41	0	5,319 or 12,803	6,698	411	263
Percentage of marked migrants			3.67						3.51			

Estimated Errors in Recovery

In analyzing the significance of such returns it is at once apparent that they represent the minimum limits. Since the marks were carefully scrutinized and checked against the scale readings only authentic recoveries have been retained. It is possible, however, that some marked adults were overlooked by the observers and that the totals are thus too low. Such condition is readily admitted, particularly in relation to the Fraser river catches of 1932. A close study of the situation throughout the area having regard to (1) the thoroughness of the recoveries in the principal canneries, (2) the relation of the total landings at other canneries to those adequately observed, and (3) the tendency of cannery employees to submit every type of mutilated fish in order to obtain a reward, indicates that the recovery could not have been less than 90 per cent of the maximum. In other words the percentage return from the sea for the first experiment in 1930 probably lay somewhere between 3.67 and 4.1 per cent.

In 1933 the methods of recovery had been somewhat refined by reason of the experiences of the previous year and the chances of error consequently rendered more limited. It is very unlikely that the error in marked individuals overlooked would appreciably raise the percentage recovery, but to embrace any such discrepancy a 5 per cent error is adopted and the true percentage, therefore, lies between 3.5 and 3.7 per cent.

Differential Mortality in Marked Individuals

There remains for consideration only the possibility of a further degree of error produced by a heavier mortality among marked sockeye than occurs naturally among unmarked ones. In such case the returns to be expected would be lower than might otherwise normally be obtained.

The question has already been raised (Foerster 1934, 1936a) in considering the returns to Cultus lake when only portions of the down-stream migrations were marked. In the two instances reported, the percentage returns of marked adults from marked migrants amounted to 0.88 and 1.34 respectively, whereas the returns of unmarked adults from the unmarked portions of the migrations represented 2.49 and 3.2 per cent. The ratios of marked to unmarked returns are 0.35:1 and 0.42:1.

To account for the disparity in percentage return three factors may be considered, namely, infiltration of unmarked adults from other areas, the straying of marked individuals to other spawning regions or a definite differential mortality among marked groups.

With reference to the first-mentioned, the occurrence among Cultus lake spawners of unmarked adults from other areas, thereby unduly weighting the proportion of unmarked in relation to marked individuals, is deemed of little significance. In 1933 when the only unmarked adults to return naturally to Cultus lake consisted of three-year fish which are generally conspicuous by their small size it was observed that 409 of a total 426 unmarked individuals measured obviously belonged to this class. The ages of the remaining 17 were in doubt.

Applying these sample proportions to the complete 1933 run, which included 3,471 adults, it is found, by calculating the fiducial limits with a confidence coefficient of 0.99 (Foerster 1936a), that from 67 to 250 unmarked adults of an age greater than three years may have occurred, the probable number being 140. Whether these were individuals from alien spawning grounds or were native spawners which had, as migrants, escaped being marked, could not be learned, but in any event the occurrence in the whole run was too slight to indicate that in the earlier experiments the infiltration of unmarked adults from other areas was responsible for the notably higher proportion of unmarked spawners in relation to the marked ones. In 1932 the unmarked group consisted of five-year fish, and though the identification of this age-class by size was not as readily made as in the case of the three-year-olds, studies of the length frequencies and of the scales from a representative sample indicated that they were mainly of the five-year group and not alien spawners.

With respect to the second possibility, namely the straying of marked fish to other areas, where they would not be observed, there is no evidence to indicate that such departure from the "parent-stream" habit occurs. Hatchery officers and fishery guardians throughout the Fraser river watershed were requested to be on the look-out for marked sockeye both in 1932 and 1933 and though the surveys were by no means thorough or complete, because of the vast territory to be covered and the difficulty in observing adequate samples from the large number of spawning areas, the fact that not a single authentic recovery was made signifies that no large body of marked sockeye penetrated to other spawning regions. Had straying of Cultus lake marked sockeye occurred it would most likely have been to adjacent lower Fraser river tributaries. On these streams hatcheries were maintained and the handling of large numbers of sockeye at these establishments would have brought to light the presence of marked individuals had they migrated there.

There remains, therefore, only the factor of differential mortality among the marked individuals, and on the data available this is held to be the one largely responsible for the lower return of marked adults when compared with that for the unmarked. Though the observations at Cultus lake at the time of marking and those of other investigators indicate that the loss due to the actual marking operation is relatively negligible there must subsequently occur in the ocean a higher degree of mortality among marked individuals by reason of the handicap under which they are placed by the removal of the fins. The fact that the returns of marked adults to Cultus lake from the present experiments, viz., 1.76 per cent for the first and 0.9 per cent for the second, are of the same order as those previously reported, namely 0.88 and 1.34 per cent, indicates that in all probability the same factor of differential mortality was operating.

Computation of Probable Return from the Sea

Accepting, therefore, 0.35:1 and 0.42:1 as representing the ratios of marked to unmarked sockeye among returning individuals, the extra mortality among marked fish lies between 58 and 65 per cent or at a mean of 62 per cent. The estimated recoveries were found to lie between 3.7 and 4.1 per cent for 1932

and between 3.5 and 3.7 per cent for 1933 or at a mean probable value of 3.75 per cent. Consequently the return, had no differential mortality occurred, would have been not less than 3.5 per cent, the lowest actual recovery made, and might have extended to as high as $\frac{100}{35} \times 4.1$ or 11.7 per cent, with a most probable value of $\frac{100}{38} \times 3.75$ or approximately 9.9 per cent.

PROBABLE RETURN FROM THE SEA, CALCULATED ON THE BASIS OF NATURAL SPAWNING

From the data acquired in the investigation of natural propagation of sockeye salmon (Foerster 1933, 1936b) it has been found that the efficiency of natural spawning in producing seaward migrants ranges from one to three per cent, with the probable value closely approximating 2.5 per cent. In other words the losses in fresh-water are in the neighbourhood of 97.5 per cent of the total calculated egg deposition. The picture for the entire life cycle can accordingly now be drawn.

From the spawning effort of one pair of sockeye salmon (the average egg content of a Cultus lake adult female having been found to be 4,500), approximately 2.5 per cent or 112 seaward-migrants are produced. Of these migrants from 3.5 to 11.7 per cent survive to return as spawning adults or from 4 to 13 fish, with a most probable value of 11 fish. For each pair of spawners, therefore, the return ranges from 4 to 13 adults or a ratio of from 1:2 to 1:6.5, the most probable ratio being 1:5.5.

These determinations are in general agreement with those of other investigators though inclining to be somewhat higher. Gilbert and Rich (1929) found for Alaska red salmon in the Karluk river that "the returns of the 1921 spawning thus far obtained indicate a probable rate of increase of three to one", whereas Holmes (1933) from researches conducted by the United States Bureau of Fisheries on the Karluk and Chignik rivers, Alaska, has shown a definite variation between spawning escapement and subsequent return of adults extending from 1:1 to 1:7 for Chignik and from 1:0.6 to 1:5 for Karluk. By combining the production for the six counted escapements in each of the two localities the ratio of escapement to return for the six broods at Chignik was found to be 1:2.5 whereas at Karluk it was only 1:1.75.

In allocating the losses occurring throughout the life history of the sockeye salmon between the fresh-water and ocean phases, however, the present findings are at variance with those reported elsewhere. For the Karluk river, according to Holmes (1933) more than 99 per cent of the mortality occurred in fresh-water. Since the egg content per female of Karluk red salmon averages 3,500 eggs, from which a maximum of 10 fish reach maturity, or a total survival of 0.3 per cent, the ocean mortality must be correspondingly low, namely about 71.4 per cent. The percentages of seaward-migrants that survived to maturity in the Karluk are recorded as ranging from 9 to as high as 25. The Cultus lake data, as outlined above, on the contrary, indicate that in fresh-water a mortality of 97.5 per cent occurs while subsequently in the ocean further losses

range from 88.3 to 96.5 per cent with a most probable value of 90.1 per cent. The total mortality throughout the life history amounts to from 99.7 to 99.9 per cent of eggs deposited with the most probable value lying at 99.75 per cent.

RELATION OF CATCH TO ESCAPEMENT

From the data of table I above, it will be observed that in the first experiment the commercial catch of 1932 amounted to 1,950 fish whereas the escapement to Cultus lake totalled 1,799 individuals, a ratio of 1:0.92 or slightly less than an even 1:1. In the second experiment, however, in 1933, the catch numbered 9,161 fish and the escapement 2,856 or a ratio of 1:0.31, approximately 3:1. The catches in the two years represented, consequently, one-half and three-quarters, respectively, of the potential spawning escapement to Cultus lake.

In Alaska, as stated by Holmes (1933) the administration of the salmon fisheries is based "upon the assumption that the number of fish produced is greater than the number required to produce them. This surplus of return over spawners is available to the commercial fishery". The practice there is to permit the capture of two red salmon for each individual counted ascending the spawning river or a ratio of 2:1.

For Cultus lake, with a ratio of escapement to return lying between 1:1 and 1:6.5, probably 1:5.5, the surplus of returning adults over spawning requirements to continue the run, generally speaking, would range from 1 to 5.5, probably 4.5, thus indicating that a similar and even higher degree of commercial exploitation would maintain the fishery. In 1932, the catch was low, approximately 1:1, thus permitting an increased seeding of the spawning beds; in 1933 it was higher, roughly 3:1, yet not endangering the continuance of the population.

SEX PROPORTIONS

Reference has been made in previous papers (Foerster 1936a, 1936b) to the seemingly very unusual predominance of adult female sockeye in the spawning runs to Cultus lake. In a subsequent study of the individuals composing the 1927-32 cycle class (now in manuscript) it was found that, although no significant difference existed between the sexes in the seaward-migration in 1929, the return of adults showed a ratio of males to females of 1:2.6.

An analysis of the sex distribution of the adults in the two experiments under review shows that while the sexes are present in the commercial catches in the ratios of approximately 1:1, those for the Cultus lake recoveries are 1:2.9 for 1932 and 1:18 for 1933. With the exception of the recoveries from Esquimalt in 1933, the returns from the fishing areas, other than the Fraser river, invariably demonstrate a varying predominance of females in the catches. For the Fraser, where gill nets are the main type of gear, the males appear to be taken in relatively greater abundance.

Consideration of the sex proportions among the seaward-migrants involved in the experiments discloses that of 123 specimens examined from the migration of 1930, 52 were males and 71 females, a ratio of 1:1.4, while for the 1931 migration even less variation occurred. The departure from a 50:50 relationship is

not sufficient in either case to be statistically significant. It is quite unlikely therefore, that unequal sex ratios among the migrants were responsible for such condition among the adults.

Clemens (1935), from data collected at the traps at Sooke, southern end of Vancouver island, reports for the sockeye runs to the Fraser river generally a normal 50:50 ratio between the sexes. Where a shortage of males does appear he attributes the condition to early maturity of the males and their precocious return to the spawning grounds the year previous. Such a situation has been found to occur at Cultus lake in but one year, 1926 (Foerster 1929b), and cannot be deemed the explanation for the many other years. Nor do the returns here reported, table I, indicate a substantial influx of three-year-old males.

The factors contributing to the predominance of females at Cultus lake must, therefore, be sought in differential mortality in the ocean among males or a fishery selective of males. The predominance of females in the trap and seine catches from Swiftsure banks to the strait of Georgia provides evidence of the former, for these types of gear should not be selective, while the gill-net catches of the Fraser river show the tendency of the latter.

There is a possibility, further, that a fishery selective of males may be carried on by Indians in the Fraser river above the commercial fishing areas and in the neighbourhood of the junction of the Fraser and Vedder rivers, up which Cultus lake sockeye pass. At the time these fish are normally caught they have begun to assume their secondary sexual characteristics, the males possessing the hooked snout and highly-arched back, by reason of which physical changes they become more readily susceptible to netting than do the rounder and more evenly-shaped females. During the fall of 1933 inspection of two catches by Indians showed that of 9 sockeye available for examination, 7 were males and 2 females. Although the numbers are too small to be of particular significance, and although no records are available concerning the total catches by Indians in these up-river areas, there is an indication that this fishery tends in some measure to reduce the proportion of males ascending to the spawning grounds.

PROGRESS OF THE RUN THROUGH THE FISHING AREAS

In figure 1 the general expanse of the area in which sockeye salmon bound for the Fraser river are captured is displayed and the division into twelve districts indicated. The numbers of fish entering the strait of Georgia through the northern passage, Johnstone strait, are not normally very great and have been but cursorily treated in this report. The migration through the strait of Juan de Fuca is the principal one and has been given chief attention. It is of interest not only from the point of view of the general route taken by the sockeye but also with reference to the relative catches in the respective districts.

In figure 3 are portrayed the total sockeye salmon landings at all canneries for the years 1932, 1933 and 1934, divided up according to the general district of capture. It might be presupposed that in a fishery devoted more or less exclusively to one river system there would be a gradual progression in the peak of the catch from the outermost fishing banks to the spawning river. Such

is but slightly indicated in the diagram for the years 1932 and 1933 and more prominently in 1934 when a sudden surge of fish in mid-season produced outstanding peaks in all localities. From studies of the rate of migration of sockeye salmon by O'Malley and Rich (1919) it has been computed that approximately 10 miles (16 km.) are covered per day in the region of the fishing areas, but later in the season the rate may be more rapid. A number of days would be required, therefore, for the fish to traverse the entire fishing ground but since

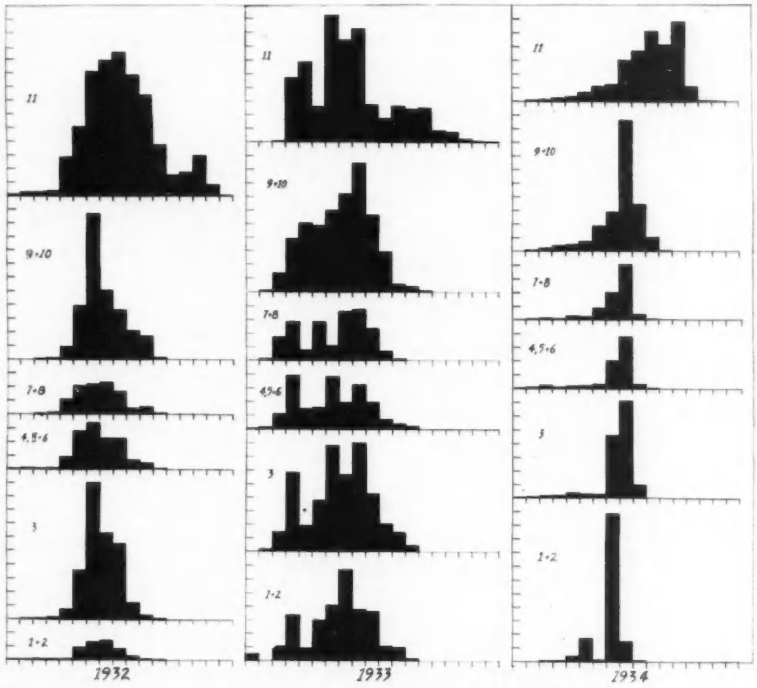

FIGURE 3. Block diagrams showing the numbers of sockeye salmon caught in each of the various fishing areas, in weekly periods, in the years 1932, 1933 and 1934. Each vertical space represents, for 1932 and 1933, 10,000 fish, and for 1934, 50,000 individuals. The horizontal spaces indicate weekly periods commencing as follows: 1932, the week ending June 25; 1933, the week ending June 19; and 1934, the week ending June 30. The fishing areas are shown in figure 1.

the run to the Fraser is made up of a large number of populations bound for different tributaries of the Fraser river system, the succession of such populations entering the fishery would be expected to mask the progression of individual ones.

The results for the marked sockeye, as tabulated in tables II and III, for the years 1932 and 1933, do not indicate, however, that any such gradual passage through the fishing areas applies even for individual migrations. From Swiftsure banks to point Roberts the recoveries were made more or less at the same periods thus demonstrating that the Cultus lake population of sockeye was

generally scattered throughout the whole area. The numbers vary from week to week and district to district, probably reflecting, in part, the fishing effort, but the peaks are by no means distinctly separated.

For the Fraser river itself, on the contrary, there is abundant evidence that the recoveries there occur much later in the season. This is naturally to be expected but the delay exhibited between the Fraser river recoveries and those in the adjacent waters of point Roberts and Boundary bay was not anticipated.

TABLE II. Recoveries of 4_2 and 5_3 marked sockeye salmon during the 1932 season, arranged according to date and area of recovery passing from Swiftsure banks inward to the Fraser river and Cultus lake. Fishing areas as in figure 1.

Date	Fishing areas						Cultus lake
Week ending	1 and 2	3	4, 5 and 6	7 and 8	9 and 10	11	
July 30	12	4	1		1		
Aug. 6	21	46	5	20	44		
" 13	18	44	8	17	46		
" 20	48	124	22	67	39	18	
" 27	57	43	16	41	117	52	
Sept. 3	58	39	13	89	36	70	
" 10	13		2	14	6	88	
" 17	1					129	
" 24	1					244	
Oct. 1						166	
" 8						26	
" 15						3	
" 22						1	
" 29						2	5
Nov. 5						13	20
" 12						5	78
" 19						1	113
" 26						1	260
Dec. 3							394
" 10							396
" 17							301
" 24							123
" 31							58
Jan. 21							17
	229	298	67	248	289	819	1765

It would appear that the sockeye tarry for a time in the estuarial waters of the river before commencing their ascent. While the peak recoveries for the Fraser are considerably later than prevail in other districts, some marked adults were taken early in the season when others of the same population were still being captured in outlying waters. Such returns again indicate that an individual spawning run may be scattered over a very wide fishing area at one and the same time.

With respect to the division of catches and marked sockeye recovered

TABLE III. Recoveries of 4_2 and 5_3 marked sockeye salmon during the 1933 season, arranged as in table II, and fishing areas as in figure 1

Date	Fishing areas						Cultus lake
Week ending	1 and 2	3	4, 5 and 6	7 and 8	9 and 10	11	
July 16	1	5			1		
" 23	3	1			0	3	
" 30	5	11			0	0	
Aug. 6	29	9	9	4	6	2	
" 13	149	73	23	81	23	10	
" 20	290	477	125	177	119	23	
" 27	294	514	297	202	468	34	
Sept. 3	869	938	217	130	35	84	
" 10	177	900	137	26	256	255	
" 17	20	64			32	319	
" 24	18	0			27	169	
Oct. 1	9	7			5	288	8
" 8	0	10			0	160	72
" 15						96	75
" 22						129	306
" 29						55	610
Nov. 5						40	254
" 12						6	216
" 19							443
" 26							679
Dec. 3							105
" 10							73
" 17							10
" 24							5
	1,864	3,009	808	620	972	1,673	2,856

between Canadian and American waters, in 1932 the catches included 652,000 and 860,000 sockeye respectively, a ratio of 1:1.3, approximately in the same proportion as the marked sockeye recovered, 819 and 1,131 respectively. In 1933, of a total catch of approximately 2,135,000, the Canadian portion amounted to 508,000 fish, producing a ratio of 1:3.2 whereas the marked sockeye returns indicated a recovery of 1 Canadian: 4.3 American. In 1934, the catches were again relatively large, the Canadian portion amounting to roughly 1,323,000 individuals and the American to 3,323,000, a ratio of 1:2.5. Of the five-year-old marked sockeye returning in that season, 140 were recovered in Canadian waters and 237 in American, a ratio of 1:1.7.

Dealing briefly with that portion of the Fraser river sockeye run which comes from the north through Johnstone strait and constitutes a considerable portion of the catch for the Quathiaski cannery, in area 12 of figure 1, this migration normally plays but a minor part in the return of sockeye to the Fraser. The landings for the Quathiaski cannery and for certain Fraser river canneries which pack sockeye shipped from the north are generally limited, being approxi-

mately 15,000, 18,000 and 9,000 respectively for the three years 1932, 1933 and 1934. That the run may, however, be of some significance at certain times is well indicated in the present year, 1936, when a most unusually large body of sockeye was observed passing down through the northern channel. Besides providing excellent fishing in Johnstone strait and adjacent waters it has reputedly contributed heavily to the fishery in the Fraser itself. The circumstances which have directed this remarkable movement of the sockeye remain undisclosed but it is clearly emphasized that in some years the migration of sockeye by this route may be a highly significant one.

Reference has been made, in reporting the results of previous sockeye markings (Foerster 1934, 1936a), to the recovery of marked Cultus lake sockeye apparently returning to the Fraser by this northern entrance. Again in collecting the marked individuals in 1933 a small but significant group of marked individuals was obtained. Their occurrence lends further evidence to the conclusion that a particular spawning population of sockeye, bound for a certain tributary stream of the Fraser river system, does not necessarily travel in a compact school nor follow the same route in its passage from the ocean to the spawning grounds.

SUMMARY

In order that the degree of survival of sockeye salmon in the ocean might be determined, seaward-migrating young from Cultus lake were marked by removal of certain fins whereby they might be identified upon their return. In 1930 the mark used consisted of the removal of the two pelvic fins, and in 1931, of the removal of the two pelvics and the adipose fin. In both years all the seaward-migrants from the lake, 104,061 and 365,265, respectively, were so treated.

In subsequent years when the fish returned as mature adults recoveries of 3.67 and 3.5 per cent respectively for the two experiments were obtained. Reference to previous marking experiments in which portions only of the seaward-migration were marked indicated that a differential rate of mortality appeared to exist whereby approximately 62 per cent of the marked migrants were lost. The loss is not attributed to the immediate effects of the marking technique since observations in the creek revealed no dead individuals and subsequent tests in which marked fish were retained in ponds for some months produced no unusual mortality, but rather to a lessened survival in the ocean.

Recognizing a 62 per cent extra mortality in the ocean among marked individuals the percentage of survival is found to range from 3.5 to 11.7 per cent, with a most probable value of approximately 9.9 per cent. By utilizing previously acquired data showing a mortality during the fresh-water phase of the life history of 97.5 per cent of eggs deposited, and assuming the average egg content of Cultus lake sockeye females to be 4,500 eggs, it is found that for each pair of spawners the return ranges from 4 to 13 adults, or a ratio of from 1:2 to 1:6.5. The most probable return approximates 11 fish providing a ratio of 1:5.5..

In 1932 the ratio of commercial catch to spawning escapement was 1:0.92

while in 1933 it amounted to 1:0.31. Since the percentage return from the sea produces a surplus over the spawning requirements this surplus may constitute the commercial catch. In 1932 the catch was low, thus providing increased seeding of the spawning beds whereas in 1933 it amounted to three times the spawning return to Cultus lake, thus limiting the extent and potential productivity of the spawning escapement.

While the sexes were approximately equally represented in the totals of the commercial catch the females predominated in trap and seine fishing except for Esquimalt (Sooke) in 1933, whereas the males were more abundantly taken in the gill nets. Females predominated heavily in the spawning run at Cultus lake, the ratios of males to females being 1:2.9 for 1932 and 1:1.8 in 1933. Since the sex proportions of the seaward migrants did not depart significantly from a 50:50 ratio, the paucity of males at Cultus must be attributed either to a heavier mortality among males in the ocean or to a fishery selective of males in up-river areas. Some evidence of the latter has been disclosed.

There is no distinct progression of the Cultus lake marked sockeye through the fishing areas. From Swiftsure banks to point Roberts the recoveries of marked individuals occur over approximately the same periods and the peaks are not definitely separable. The recoveries in the Fraser river were made principally in the latter part of the season but scattered returns were obtained throughout the summer. A notable delay was found to occur between the recoveries at point Roberts and Boundary bay and those in the Fraser, thus indicating that the fish did not pass immediately into the river and ascend to the spawning grounds. Recoveries were also received from Quathiaski cannery indicating that Cultus lake sockeye also entered the strait of Georgia from the north. Evidence is thus presented to show that the Cultus lake population does not enter from the ocean in a compact school and does not necessarily confine itself to a single route.

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Oceanography of Nootka Sound

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ABSTRACT

Characteristic tidal circulation in a canal, a fiard and a fiord is shown and it is indicated that the depth of tidal circulation in those inlets having a threshold probably varies inversely as the temperature of the circulating waters. A series of tidal observations indicated the presence of a semi-daily tide cycle in the surface waters and a daily tide cycle in the deep waters of the sea adjacent to the sound. Evidence of the upwelling of deep ocean water along the coast is shown by the oxygen content. A zone of photo-synthetic activity between 3 and 6 metres depth is outlined by the occurrence of chemical anomalies.

An investigation of the physical and chemical properties of the waters of the sea and the three inlets directly contiguous to Nootka sound on the west coast of Vancouver island was carried out from July 25 to 27, 1933. For this work the Canadian Hydrographic Survey vessel, C.G.S. *Wm. J. Stewart*, was generously made available by the Department of Marine.

The study involved the sampling of a column of water at each of eighteen stations, four (2231 D-G) in a line magnetic south (205° True) from Nootka light, one (2231 M) tidal station opposite Nootka light, four stations (2233 I-L) in Tasis canal, three (2234 A-C) in Tlupana inlet, and three (2235 H-O) in Muchalat inlet. The chart (figure 1) shows the location, configuration and area of the waters, and the positions of the stations.

The data obtained are tabulated, and illustrated as cross sections of the various areas along the lines of the stations, showing isometers of the concentration of the various constituents of the water. The horizontal scale of the cross sections is natural and the depth scale is logarithmic as shown. This latter convention enables the epithalassal phenomena to be shown in greater detail than would be possible with a natural depth scale.

The observations were made at 1, 2, 4, 6, 10, 20, 30, 50, 100, 200, and 400 metres, or at as many of these intervals as the depth of the water at any station would permit, and consisted of time of observation and tidal phase, the range of the tide in metres, depth in metres, temperature in degrees centigrade, chlorinity as Cl $^0/_{00}$, density as σ_t (Knudsen 1901), also analyses for hydrogen ion concentration as pH, dissolved oxygen as milligram-atoms per litre and as per cent of saturation, phosphate phosphorus and silicate silicon as milligram-atoms per litre, and at several stations a sample of the bottom was taken with a clam-shell dredge.

During the progress of the survey the weather was consistently fine. In the morning the wind was usually light and easterly, and after a noon calm the direction changed to westerly, falling again to calm after dark. The sky was clear throughout the entire period.

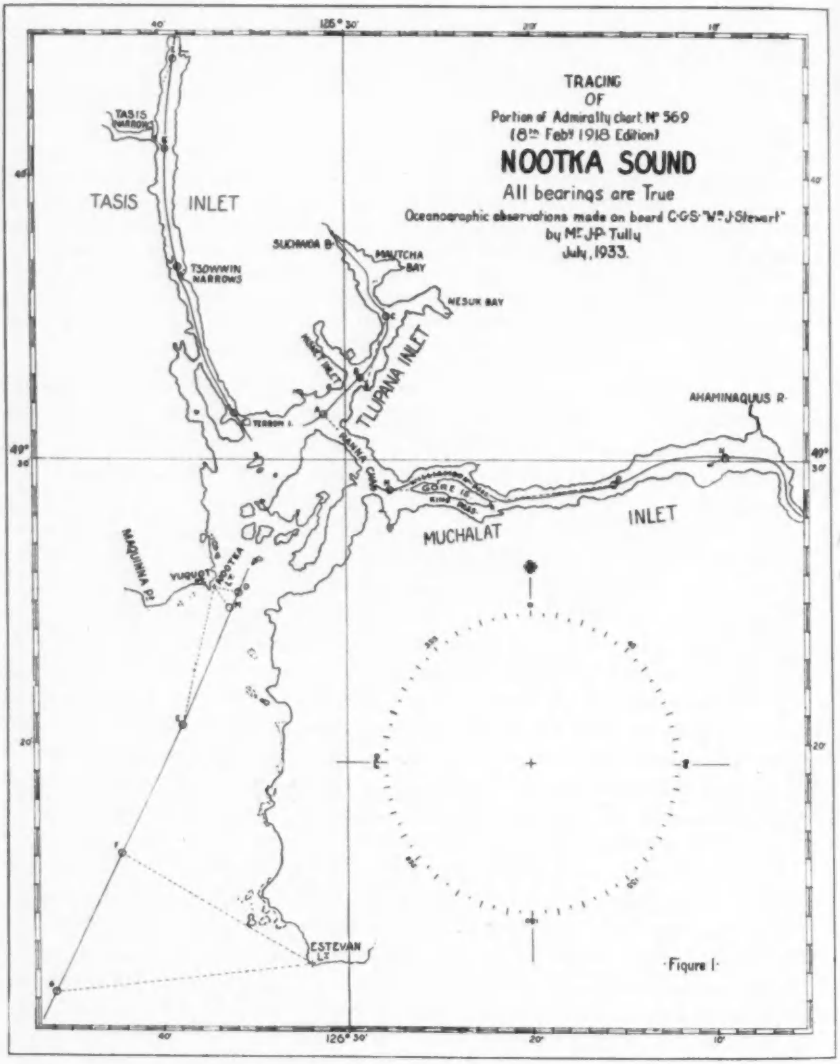

FIGURE 1.

July 25, 1933

Station	Bearing	Distance (kilometres)	To	Time	Tide ht. (metres)[1]
2231-D	282°	1.48	Nootka Lt.	8:45- 8:55	0.67
2231-E	13°	9.25	Nootka Lt.	9:48- 9:56	1.07
2231-F	120°	14.45	Estevan Lt.	10:44-10:54	1.65
2231-G	84°	16.88	Estevan Lt.	11:52-12:02	2.26

[1]Tide tables of the Pacific Coast of Canada for the year 1933 (Dept. of Marine).

The range of the tide was 2.65 metres in 6 hours 17 minutes between 8:13 and 14:31 o'clock. All the observations were made on a neap flood tide, and the data are presented to show the trend of oceanographical conditions at this time. It was observed that the flow of the surface waters was northwestward along the line of the stations. This is not strictly true over the whole of this area nor for the deeper waters, but indicates the general direction of the movement.

PHYSICAL OBSERVATIONS

Due to the influence of land drainage, the surface sound waters are fresher (figure 2, B) than the coast waters, whose normal density is greater than 24.50, and their influence is noticeable in the surface layers for about 25 kilometres south of Nootka light, the two bodies merging gradually without any evidence of a thermocline. This indicates that there is considerable tidal circulation and that the direction of the flow of the two phases of the tide is not reciprocal outside of the sound.

Since the Ferrelian forces of the earth cause all currents to veer to the right in the northern hemisphere (Sandstrom 1918) the flood tide current coming from the south along the general coast-line of Vancouver island tends to hold closely against the coast at all points, following parallel and close to the western shore of Estevan peninsula, while the ebb, being reciprocal in direction but obeying the same laws, tends to swing towards Maquinna point and flow westward. Thus the ebb tide waters are carried out of the path of the flood, and the latter brings coast water into the sound, at the same time forcing the former still farther westward. This movement is against the prevailing winds which tend to cause a surface drift to the eastward so that, as a resultant of the two influences, the Nootka sound waters are distributed over the whole surface of the sea in this locality.

At station D (see figure 2, A) there was a relatively sharp fall in temperature and a rise in density at 4 metres depth, but at no other place was the transition from the surface layers to the deep water marked by any such change in properties. This station was observed at the time of low water slack and exhibits the limits in variability in the physical and chemical characteristics due to the ebb tide flow. The other stations in the line were observed as the flood was further advanced and indicate that the water was warm at the entrance to the sound, south of which there was a body of colder water, 10° to 12° C., and south of the latter and apparently overflowing it, was another body of warmer water at 12.6° C., which reached a maximum depth of 11 metres at station G. There was a tendency for this phase of the tide to crowd the surface layers back towards the sound, and to have its maximum effect between 3 and 15 metres depth.

TABLE I

Depth (metres)	Cl°/₀₀	Temp. °C.	Density in situ σ_t	Diss'd oxygen Mg. A/L	Diss'd oxygen %sat'n.	pH	SiO_3 Mg. A/L $Si \times 10^3$	PO_4 Mg. A/L $P \times 10^3$
				Station 2231-D				
1	14.98	14.7	19.96	0.6210	116.8	8.3	5.3	0.69
2	15.18	14.2	20.34	.6190	115.3	8.2	5.3	0.73
4	17.42	10.4	21.33	.6810	121.2	8.2	9.2	1.07
6	17.70	9.7	24.67	.6320	111.3	8.1	13.7	1.17
10	17.78	9.05	24.88	.4500	78.5	8.0	17.2	2.02
20	17.92	8.5	25.16	.3505	60.5	7.8	28.4	2.38
30	17.93	8.4	25.19	.3083	53.0	7.9	37.3	2.73
50	18.14	8.0	25.54	.3005	51.5	7.9	38.4	2.65
100	18.24	7.75	25.73	.2495	42.6	7.8	38.4	2.85
				Station 2231-E				
1	17.40	11.6	23.92	0.6795	124.0	8.3	5.2	0.99
2	17.41	11.6	23.93	.6430	117.5	8.3	7.1	0.90
4	17.43	11.1	24.05	.6600	119.3	8.2	7.4	0.80
6	17.73	10.9	24.51	.6325	114.2	8.1	14.0	1.00
10	17.79	9.7	24.77	.4595	81.1	8.0	16.2	1.40
20	17.93	9.25	25.07	.4160	73.1	7.9	28.0	1.51
30	18.08	8.3	25.41	.3250	55.9	7.9	34.1	1.90
40	18.19	7.9	25.63	.2760	47.2	7.9	38.2	2.01
				Station 2231-F				
1	17.44	12.1	23.88	0.6605	122.0	8.4	8.7	1.16
2	17.44	12.1	23.88	.6750	124.6	8.4	9.1	1.06
3	17.44	12.0	23.90	.6500	119.9	8.4	9.2	1.01
4	17.54	11.6	24.12	.6440	117.8	8.4	8.5	1.22
7	17.72	10.8	24.51	.5465	98.7	8.1	13.1	1.55
17	17.91	9.0	25.08	.3855	67.3	7.9	13.5	2.16
27	18.05	8.25	25.38	.2575	44.2	7.8	14.5	4.33
				Station 2231-G				
1	17.67	12.6	24.11	0.6180	115.7	8.4	....	0.82
2	17.68	12.6	24.12	.6200	116.0	8.4	....	0.75
4	17.68	12.6	24.12	.6200	116.0	8.3	....	0.80
6	17.68	12.6	24.12	.6020	112.8	8.2	....	0.80
10	17.73	12.5	24.23	.5120	95.5	8.2	....	0.87
20	17.94	9.4	25.06	.4690	79.9	8.1	....	1.68
30	18.10	8.15	25.46	.3715	63.0	8.0	....	2.12
50	18.27	7.5	25.80	.2922	49.6	7.8	....	2.57
100	18.80	6.05	26.75	.0683	11.3	7.7	....	2.86

In the layers between 15 and 40 metres depth the temperature fell and the density rose very regularly, indicating regular circulation. The waters below 40 metres were divided; those between the banks (figure 2) and the sound showed a marked lack of vertical stability, while those to seaward continued to become regularly colder and more dense with increasing depth. There is no evidence in this cross section of any connection between the inner and outer deep body

of water at this season of the year, although in the winter when the surface becomes chilled, the vertical column of water will tend to become homogeneous, stability will approach a minimum, and circulation may be accomplished (Hutchinson, Lucas and McPhail 1929).

Physically, the waters were divided into three zones, first the epithalassal, which may be arbitrarily defined as being that in which the temperature was above 10° C. and the density less than 24.50; secondly the coast water between this and 40 metres depth, in which the temperature dropped to 7.8° C. and the density rose to 25.50; and below this a body of deep sound water inside, and deep coast water outside the banks, which apparently had no circulatory connection between them.

CHEMICAL OBSERVATIONS

The degree of saturation with dissolved oxygen (figure 2, C), was high near the surface, increased to a maximum at about 3 metres depth, and decreased to saturation at the contact of the coast water zone, all the supersaturation occurring in the epithalassium.

This condition of supersaturation is common at this season when the water is still or circulating quietly and is indicative of a flourishing phytoplankton population (Gran and Thompson 1930). The vagaries shown in the section may have been due to the uneven distribution of plankton colonies in the neighbourhood.

The oxygen content fell regularly through the coast water layer, from saturation, at the contact with the epithalassal zone, to 48 per cent at 40 metres and in contact with the banks. This gradation was very even over the whole section and is further indication of the uniformity of movement in this part.

The deep layers showed decreasing saturation but with different characteristics in the two locations. In the deep sound water the saturation dropped to 42 per cent at 100 metres, while at corresponding depth in the deep seaward water the value was 11.3 per cent. The fact that the deep layer inside the banks was carrying more oxygen is further indication of a lack of connection between the two zones and shows that the inside water had been in contact with the surface more recently than the outer. It is most probable that active circulation is set up between the coast water zone and the inside deep water by the tidal currents in the cold seasons when the former is more dense and so prevents the latter from becoming stagnant.

The oxygen gradient in the Pacific ocean is characterized by a decrease from saturation at the surface, to between 3 and 12 per cent saturation at about 700 metres depth (Moberg 1930). The low saturation observed at station G may be explained by the theory that deep ocean water upwells along this coast due to the prevailing westerly winds (McEwen 1912).

The pH (figure 2, D) was very regular, falling rapidly from a maximum of 8.4 at the surface to 8.1 at the contact of the epithalassal and the coast water zones. Then it fell more slowly to 7.8 at 40 metres in contact with the bottom over the bank, and to 7.7 in the deep seaward water.

The distribution of dissolved phosphates and silicates (figure 2, E and F)

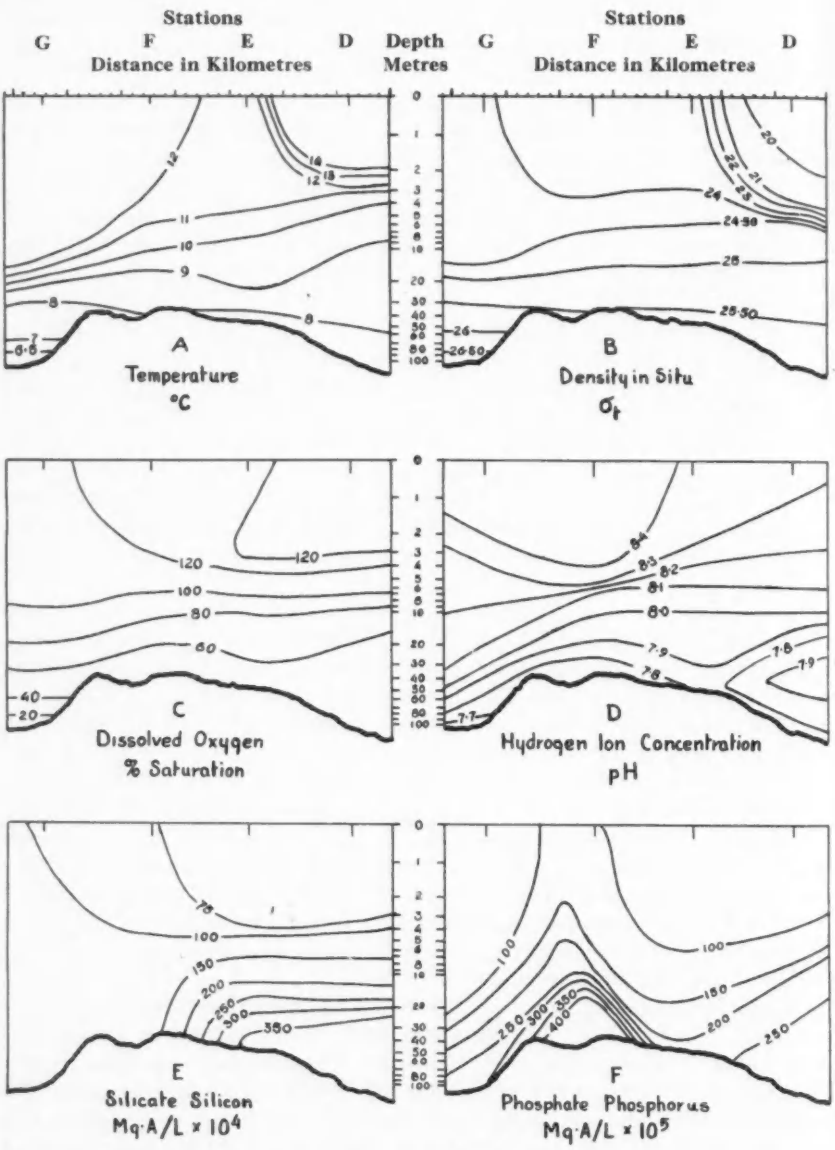

FIGURE 2. Graphical representation of the distribution of the physical and chemical properties of the water, in the sea adjacent to Nootka sound, along the line of the stations 2231-D, E, F, G.

was generally similar, the concentration of both increasing from very low values at the surface near the entrance to the sound, to normal values outside the zone of influence of the sound waters, and at the bottom of the epithalassal layer. They were both very evenly distributed throughout the coast water zone, but in the deep water, the silicates were more concentrated inside the banks than outside, while the phosphates were just the reverse.

The drainage waters from Vancouver island have a very low pH (the values being of the order 6.0 to 7.0), a very high silicate content (approaching a concentration of 9×10^3 milligram-atoms per litre in some cases) and no dissolved phosphates. In such sounds as this where the waters are confined, the concentration of dissolved silicates is higher, the pH is lower, and the phosphates less than would be expected in open waters with similar concentrations of the other dissolved constituents.

These tests were undertaken to determine the relative abundance of these two nutritive elements, and it appears that the available supply was nearly exhausted in the zone in which the greatest photo-synthetic activity was indicated by the observations of dissolved oxygen. It is also apparent that the deep waters of the sound carried a higher silicate and a lower phosphate concentration, than the ocean water adjacent to the coast, and the samples from both locations showed higher concentrations of these elements than are commonly found in the strait of Georgia at similar depths (Lucas 1929).

TIDAL OBSERVATIONS

At the entrance to Nootka sound, observations were made at each slack water in one lunar day, to determine the limiting effects of the tidal flow, and permit some estimation of the rate of interchange of the waters in the sound with those in the adjacent sea.

In each instance the observations were made at the standard depths and at the time of observed slack water at the surface.

STATION 2231-M

Observation	Date	Time of slack water		Height
		Predicted	Observed	(metres)
I	July 26	15:00	15:18-15:28	3.29
II	" 26	21:04	21:15-21:38	1.34
III	" 27	2:50	3:07- 3:18	3.21
IV	" 27	9:15	9:26- 9:42	0.91

During the observations the weather was fine, the tides were neap and the maximum observed surface velocity was approximately 2 kilometres per hour.

The scales of the sections (figure 3, A to F) are so arranged that time is the horizontal component, plotted on a natural scale, and the depth is vertical, and on a logarithmic scale as in figure 2.

PHYSICAL OBSERVATIONS

In the section showing the variations of density (figure 3, B) there is distinct evidence of a semi-daily cycle of circulation in the epithalassal zone. On the

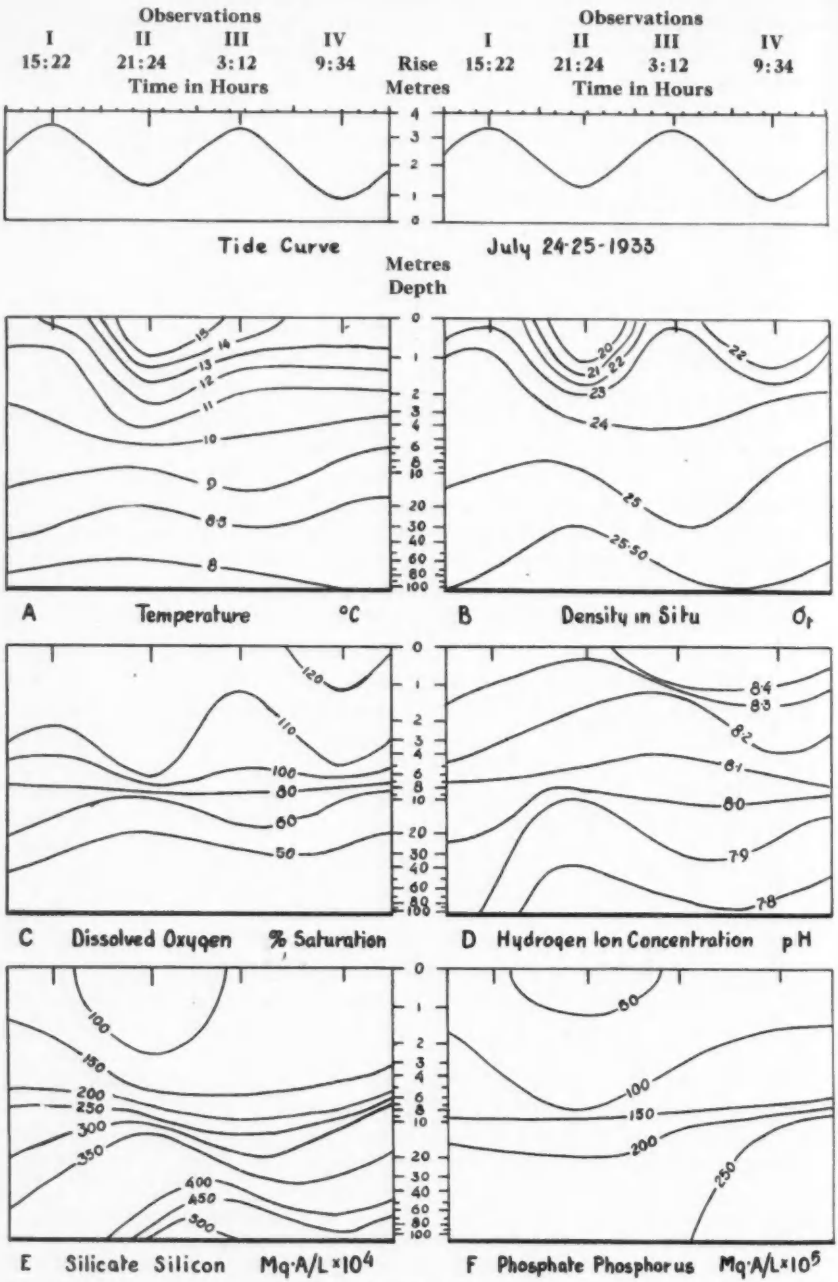

FIGURE 3. Graphical representation of the distribution of the physical and chemical properties in the sea water during the twenty-four hour period between noon July 24 and noon July 25, 1933, at station 2231-M at the entrance to Nootka sound.

flood tide, water of density 24.00 was evident at the surface, and on the flow of the ebb the water freshened to a minimum density of 19.00. The flood brought back the denser water and the cycle was repeated.

TABLE II

Station M

Depth (metres)	Cl°/₀₀	Temp. °C.	Density in situ σ_t	Diss'd oxygen Mg. A/L	Diss'd oxygen % sat'n.	pH	SiO_3 (Mg. A/L) Si×10³	PO_4 P×10³
				Observation I				
1	17.37	10.9	24.00	0.6320	113.5	8.3	13.7	1.00
2	17.42	10.5	24.15	.6200	110.7	8.2	15.3	1.00
4	17.66	9.9	24.59	.5745	103.3	8.2	15.8	1.02
6	17.81	9.55	24.85	.5130	90.3	8.1	22.4	1.23
10	17.90	9.1	25.04	.3855	67.3	8.0	28.4	1.57
20	17.99	8.8	25.21	.3232	56.1	8.0	30.5	2.06
30	18.07	8.5	25.37	.2940	50.9	8.0	34.4	2.18
50	18.09	8.2	25.45	.2678	46.0	7.9	37.4	2.22
100	18.20	7.8	25.66	.2485	42.4	7.9	39.1	2.14
				Observation II				
1	14.45	15.2	19.14	0.5860	110.5	8.2	5.3	0.48
2	16.53	12.5	22.54	.6355	116.9	8.2	8.4	0.66
4	17.48	11.1	24.12	.6395	116.6	8.1	13.1	0.77
6	17.61	9.9	24.51	.6305	111.7	8.1	15.8	0.78
10	17.90	8.9	25.07	.3550	61.8	7.9	28.6	1.77
20	17.99	8.5	25.26	.2925	50.5	7.8	38.4	2.00
30	18.15	8.3	25.52	.2768	47.6	7.8	38.4	2.13
50	18.15	8.1	25.55	.2715	46.6	7.8	39.1	2.13
90	18.28	7.8	25.78	.2417	41.2	7.8	52.5	2.24
				Observation III				
1	16.64	13.2	23.56	0.6005	112.0	8.4	12.5	
2	16.81	11.0	23.20	.5940	106.2	8.1	14.0	
4	17.26	10.6	23.90	.5850	104.1	8.1	14.9	
6	17.56	9.6	24.50	.5490	96.5	8.1	14.9	
10	17.61	9.2	24.62	.4195	72.0	8.0	20.4	
20	17.70	8.8	24.81	.3280	56.8	7.9	32.0	
30	17.82	8.5	25.03	.2880	49.4	7.9	35.4	
50	17.97	8.2	25.29	.2735	46.9	7.8	42.8	
90	18.19	7.8	25.65	.2477	42.2	7.8	49.1	
				Observation IV				
1	15.74	12.9	21.37	0.6800	124.8	8.4	13.6	0.93
2	17.12	10.7	23.69	.6660	117.8	8.3	13.6	1.05
4	17.65	9.95	24.56	.6495	115.0	8.2	15.4	1.11
6	17.75	9.0	24.85	.6050	105.1	8.1	15.9	1.26
10	17.87	9.0	25.02	.3480	60.7	8.0	28.5	2.39
20	18.03	8.5	25.31	.3025	52.3	7.9	35.5	2.76
30	18.07	8.3	25.40	.2872	49.4	7.9	34.4	2.92
50	18.07	8.25	25.41	.2735	47.0	7.8	37.9	2.80
90	18.14	8.2	25.51	.2520	43.3	7.8	47.4	2.57

The cycle of the temperatures (figure 3, A) exhibited a similar phenomenon, the cold water appearing on the flood, and the warm water from the inlets on the ebb, except on the morning ebb tide (observation IV), when the surface did not become as warm as on the afternoon ebb of the previous day (observation II), which was probably due to the lack, during the night, of the warming influence of the sun.

Between 7 metres depth and the bottom there was a zone characterized either by a semi-daily tidal oscillation directly opposite to that in the epithalassium, or a daily oscillation. The temperature and chlorinity data can be interpreted in either way, but those of the chemical properties indicate the daily type of tide.

The data as a whole yield more readily to the assumption of a daily rather than a semi-daily tide in the deep waters, and have been illustrated as such.

If the characteristics of the two cycles are compared (figure 3, A and B), and assuming that the surface three metres represents the purely semi-daily type and the isometer of density 25.50 or of temperature 8.2° represents the purely daily type of tide, it will be seen that, at the time of observation, the period of high water in the daily tide occurred close to the time of low water in the semi-daily zone, and that low water in the former approached the time of high water in the latter. There is a tendency for each to balance the other and at the boundary of these two distinct tidal oscillations there was a layer partaking of the character of both, in which there was very little change in the temperature or in the density.

It is suggested that this apparent dual circulation may possibly be connected with the two major tidal forces, one of which causes a diurnal and the other a semi-daily tide, both of which are present on this coast, giving rise to the resultant tides as usually observed (Marmer 1932).

This may offer an explanation for the variation between the phase and direction of the tide as noticed at many points near deep water. The rise and range of the tide are resultants of all the forces acting, but it is a well known fact that the times of ebb and flow do not generally correspond to the times of the fall and rise of the tide. If, as seems to be indicated, the tidal forces are located in different levels, then the run would follow the laws of the surface (semi-daily) tide only, and the tide might flow in the flood direction while the level of the water is falling, or vice versa. Further investigation of this anomaly is in progress.

CHEMICAL OBSERVATIONS

The reciprocal nature of the tidal currents in the entrance to Nootka sound is further indicated by the distribution of dissolved oxygen (figure 3, C). In the surface layers the degree of saturation increased on the ebb tide as the warm sound waters were carried past the station, and decreased on the flood as these were carried back, and with them the colder waters from the sea. The waters of the epithalassium were supersaturated with oxygen on both tides, but it is noticeable that the saturation did not show any tendency to increase with depth to about 3 metres, as was the case at all places where the movement of the water was small. This is a further indication of a strong current past this station.

Throughout the whole section the isometers of oxygen saturation closely paralled those of temperature and density, each zone having its own characteristic values.

The surface waters ebbing from the sound (figures 3, D) were more acid than those flooding from the sea, which follows naturally from the drainage of th characteristically acid land water into the sound.

At observation IV (figure 3, D) the pH did not fall at the surface as would be expected, probably due to the lessened flow of the rivers into the sound during the night. This deduction has further support in the value of the surface density which did not fall as low, and the temperature which did not rise as high as on the low water of the previous evening, although the fall of the tide was a quarter of a metre greater.

The distribution of the dissolved silicates and phosphates was evidently random (figure 3, E and F) only the concentration along the dividing line between the epithalassium and the deep water showing any continuity, possibly due to the constant mixing of tidal currents at this level.

The concentration of these inorganic nutrients is sufficient for the support of marine plankton (Atkins 1926), and only at the surface during observations II and III was there any indication of partial depletion.

The concentrations of both the phosphates and the silicates were found to be of the same order below the epithalassium in this area (section D to G) as in the strait of Georgia at similar depths (Lucas 1929).

INLETS

There are three inlets opening into Nootka sound, each being characteristically different from the others in some respect. Tasis inlet is a canal, Tlupana inlet is a fiard, and Muchalat inlet is a fiord (Carter 1934).

Each was investigated oceanographically and the peculiarities resultant from the geographical configurations were observed.

Tasis Inlet

Except for the entrance of Hecate channel at Tasis narrows, this canal has all the characteristics of a true fiord. The mountains are between 2000 and 3000 feet (600 to 900 metres) high, the sides are precipitous, and there is a definite threshold at Tsowwin narrows inside of which greater depths are evident than are found outside.

July 26, 1933

Station	Time observed	Height of tide (metres)
2233-I	8:15- 8:38	0.73
2233-J	9:30- 9:40	0.79
2233-K	10:22-10:38	1.03
2233-L	11:18-11:37	1.43

The range of tide was 2.56 metres in 6 hours 16 minutes between 8:44 and 15:00 o'clock.

TABLE III

Depth (metres)	Cl°/₀₀	Temp. °C.	Density in situ σ_t	Diss'd oxygen Mg. A/L	Diss'd oxygen %sat'n	pH	SiO_2 (Mg. A/L) Si×10³	PO_4 (Mg. A/L) P×10³
				Station 2233-I				
1	13.56	14.2	18.10	0.6485	118.3	8.1	13.8	0.48
2	14.39	13.0	19.47	.6170	111.0	8.1	14.2	0.53
4	17.02	9.7	23.72	.4850	84.8	8.3	31.6	1.29
6	17.21	9.4	24.03	.4510	78.7	8.2	34.1	1.34
10	17.84	8.95	24.99	.3840	66.9	8.0	34.5	1.72
20	17.91	8.40	25.17	.3060	52.6	7.9	43.5	1.87
30	18.00	8.3	25.31	.2800	48.1	7.9	49.2	1.97
50	18.06	8.1	25.42	.2575	44.1	7.8	52.0	2.17
				Station 2233-J				
1	12.56	15.2	16.52	0.6600	121.7	8.4	14.1	0.61
2	13.35	14.6	17.73	.6960	128.1	8.4	14.1	0.51
4	16.16	11.3	22.24	.7140	127.1	8.4	18.8	0.66
5	16.84	10.3	23.36	.6485	114.2	8.3	24.1	1.14
6	17.14	9.7	23.87	.5205	91.2	8.2	25.0	1.87
15	17.60	8.9	24.66	.3355	58.2	8.0	36.0	2.10
25	17.76	8.6	24.93	.3075	53.0	7.95	45.0	2.01
45	17.85	8.4	25.08	.2802	48.2	7.9	45.0	2.04
95	17.88	8.35	25.13	.2788	47.9	7.9	49.0	2.00
				Station 2233-K				
1	15.53	15.8	20.50	0.6705	129.9	8.4	14.1	0.33
2	16.57	12.2	22.65	.6900	126.1	8.4	15.0	0.52
4	16.65	10.8	23.02	.6440	114.3	8.3	18.1	0.69
6	17.26	9.9	24.02	.5947	104.5	8.1	26.8	0.73
10	17.55	8.9	24.58	.3407	59.0	8.0	47.4	1.33
15	17.60	8.9	24.66	.3275	56.7	8.0	42.1	1.42
25	17.72	8.6	24.87	.2863	49.4	8.0	42.1	1.54
45	17.84	8.4	25.07	.2815	48.3	8.0	42.1	1.45
95	17.88	8.3	25.13	.2268	38.9	8.0	41.5	1.50
185	18.02	8.5	25.31	.0391	6.8	7.9	65.7	2.48
				Station 2233-L				
1	12.64	14.6	16.75	0.7030	128.0	8.5	14.6	0.47
1.5	15.09	14.3	20.19	.7850	146.4	8.5	15.6	0.50
2	15.20	13.2	20.56	.7500	137.1	8.5	16.8	0.50
4	16.92	11.3	23.30	.6950	125.0	8.4	19.5	0.74
6	17.20	10.0	23.82	.6315	110.2	8.4	25.0	0.95
15	17.51	9.0	24.51	.4095	71.0	8.0	31.8	1.68
25	17.66	8.5	24.80	.3220	55.3	7.9	35.9	1.93
45	17.79	8.3	25.01	.2705	46.5	7.9	42.1	2.18
95	17.93	8.3	25.21	.2372	40.7	7.8	42.1	2.33

PHYSICAL OBSERVATIONS

The epithalassal zone in Tasis inlet had a density less than 23.00 (figure 4, B) and a temperature greater than 10° C. (figure 4, A). The distribution of these properties indicate, that part of this layer extended into Nootka sound, but it

is evident from comparison with station M that the continuity did not extend as far as Yuquot point. This zone had a regular density and temperature gradient, and was bounded by a slight thermocline at 3 metres depth at station I, at 4 metres at station J, at 2 metres at station K and at 3.5 metres at station L. At the latter place there was a body of comparatively fresh water extending to a depth of 1.5 metres which evidently had its source in the Tasis river at the head of the inlet.

The shape of the isometers of density and temperature indicate that on the flood tide original surface layers were forced towards the middle of the canal where they became isolated to form a "lake".

Below the epithalassium north of Tsowwin narrows there was a layer of water about 100 metres in depth, in which the density rose from 23.00 to 25.30 and the temperature fell from 10° C. to 8.3° C., and which was continuous with the waters of Nootka sound and Hecate channel between 3.5 and 50 metres depth. This constituted the circulatory zone of the inlet, the tide flooding from Nootka sound and Hecate channel simultaneously, and the water being drawn from the epithalassium and the upper part of the coast water zone.

It is evident that when the waters borne on the tidal current entered Nootka sound, they passed below the less dense water entering from the inlets on the ebb tides. The main tidal current was several metres below the surface, carrying the circulatory waters quietly from the mouth of the canal to Tsowwin narrows, where they rose to the surface and forced the epithalassal waters of the canal back into the main reach. Once over the threshold, the tidal waters again passed below the surface layers to occupy the zone between 8 and 100 metres depth, the velocity becoming less, and the current finally ceasing as it met the influence of the currents from Tasis narrows, which were similar in character but had a much smaller flow than those from the south.

The main flood tide current from Hecate channel entered the canal between the surface and 50 metres depth and divided in two, part moving up the canal to the head, and part towards Tsowwin narrows. From the data it would appear that the flood occurred at approximately the same time in Tasis as in Tsowwin narrows, and this water was similar in the range of observed characteristics to that from Nootka sound, and on entering the canal it descended below the surface layers in the main reach throughout the circulatory zone.

On the flood tide the surface current is regular and northwards from Terron island to Tsowwin narrows, where the maximum velocity of 3 to 5 kilometres per hour is reached at half tide. The current then diminishes rapidly and in a few kilometres ceases altogether. Still water prevails to within a kilometre of Tasis narrows where a very weak southerly flowing current may be encountered, and from Tasis narrows to the head of the inlet there is little or no northward flow. These opposing flood tide currents tend to crowd the surface layers between Tsowwin and Tasis narrows so that they form a "lake" (figures 4a and 4b), reaching a maximum depth of 8 metres between the two narrows.

The tendency of the fresh water from the Tasis river to spread over the surface induces a continuous flow towards the mouth which is not great enough

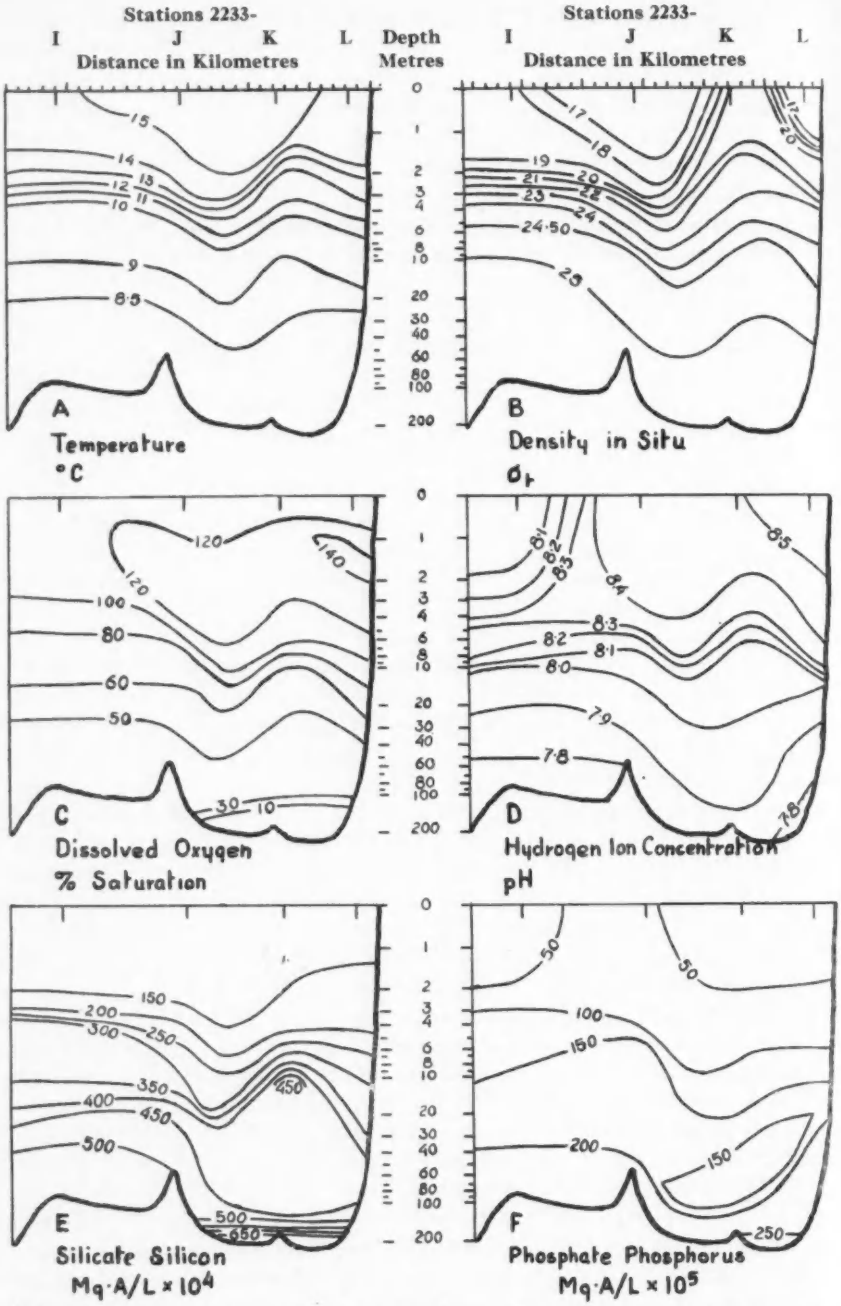

FIGURE 4. Graphical representation of the distribution of the physical and chemical properties of the waters in Tasis inlet.

to oppose the flood tide, but detracts from its velocity and adds to that of the ebb. (Sandstrom 1918).

On the ebb tide, the waters from the head drain in a southerly direction leaving the canal by Tasis narrows, and for about 2 kilometres south of this the flow is northwards. The remaining waters flow towards Nootka sound, the velocity increasing to a maximum at Tsowwin narrows, past which it moderates and the flow becomes regular. On this tide the epithalassal zone extends toward Nootka sound and to a less extent toward Hecate channel.

The properties of the water below 100 metres in the deep basin between Tsowwin narrows and the head of the inlet did not correspond with the Nootka sound waters at similar depths. The density of the former was significantly less throughout the vertical range and did not reach as great a value at 185 metres as the latter at 50 metres. The temperature fell regularly to 8.1° C. at 50 metres south of the threshold but north of this the minimum temperature of 8.3° C. was reached at 95 metres, and the water became warmer with increasing depth, reaching 8.5° C. at 185 metres.

These properties distinguished the two bodies of water and indicated that there was no connection between them. Those from the sound below 55 metres did not circulate in the canal north of the threshold in Tsowwin narrows, and those below 100 metres in the deep basin did not have any connection with the sound, and in all probability this zone is stagnant at all times.

CHEMICAL OBSERVATIONS

The chemical properties of the epithalassal zone confirmed the deductions from the physical properties of these waters.

The saturation with dissolved oxygen rose, between Tsowwin and Tasis narrows, from 110 per cent at the surface to 120 per cent between 1 and 4 metres depth, and then fell to 100 per cent at the thermocline (figure 4, C). This zone of supersaturation had a high pH, 8.3 to 8.4 (figure 4, D), and a comparatively low silicate and a very low phosphate content (figure 4, E and F).

The oxygen saturation between 1.5 and 5 metres depth in the middle of the canal, in conjunction with the relatively low concentration of silicate and the deficiency of dissolved phosphate beyond the influence of fresh water, indicated the likelihood of great photo-synthetic activity which in turn leads to the assumption of a high phytoplankton content (Gran and Thompson 1930).

The influence of the surface sound waters was noticeable in the distribution of dissolved oxygen and pH near the entrance of the canal and at Tasis narrows. The flooding waters, which force the surface waters of the inlet toward the head and middle of the canal, did not have as high a pH or as great a subsurface supersaturation with dissolved oxygen as the canal waters, and may be assumed to have had a lower plankton content.

The highest oxygen content was in the head waters of the canal and was accompanied by the highest pH despite the acidity of the inflowing fresh water. The silicates in this zone were of sufficient concentration for plankton development but not greatly in excess of the other surface values as would be expected. It is customary to associate unusually high pH values in sea water with super-

saturation of dissolved oxygen, and partial or complete exhaustion of the silicate and phosphate resources, and consequently with great phytoplankton development (Brandt 1929), which would lead to the consideration of this water as having had the greatest phytoplankton activity to be found in the canal.

Through the circulatory zone the oxygen concentration fell from 100 per cent to 40 per cent, the pH from 8.3 to 7.9, and the concentration of dissolved phosphates and silicates rose to the normal values as found in the coast waters of corresponding depths.

The continuity of the circulation between the level of the thresholds and 100 metres was indicated by the normal oxygen content of these waters which, similarly to the density and temperature, was continuous with that in the circulatory zone south of Tsowwin narrows.

If these values of the chemical properties, which may be assumed to be characteristic of the tidal waters, are compared to those found at station M, the waters in the canal on the flood tide may be identified with the epithalassal and circulatory zones in the coast water on the corresponding tide.

The waters south of Tsowwin narrows were chemically and physically similar in every respect to the sound waters of corresponding depths as shown at stations D and M.

Below the circulatory zone in the main basin of the inlet the concentration of dissolved oxygen fell from 45 per cent at 100 metres to 6 per cent at 200 metres depth, the pH fell gradually to 7.8, and the concentrations of phosphates and silicates rose to values similar to those in the deep sound waters.

A clamshell dredge sample, taken at station K, showed the bottom to be a green clay overlaid with a black organic mud, which had a strong odour of hydrogen sulphide, and showed no evidence of life, although there were bits of wood and other organic debris present.

It has been shown in the investigation of Saanich inlet (Carter 1934), that the presence of a threshold such as at Tsowwin and Tasis narrows, causes the water below its level to be sheltered from the circulatory influence of the tides, so that it becomes stagnant, the normal oxygen content becomes exhausted and dead plants and animals falling to the bottom decompose slowly giving off unoxidized compounds such as hydrogen sulphide.

It may be supposed that the tidal influences assure a regular circulation, which may be characterized, in view of the necessarily reciprocal nature of the flow, as a flushing action which appeared to be effective to a depth of 100 metres as far as Tsowwin narrows. North of this the lower limit of the flow was at approximately the same depth, but the waters between the epithalassium and 100 metres in the canal were continuous with those above 50 metres south of Tsowwin narrows, from which it may be reasoned that the main effort of the tidal current was between the epithalassium and the level of the thresholds, that is, between 4 and 55 metres at Tsowwin narrows, and between 1.5 and 55 metres at Tasis narrows.

Consideration of the distribution of the physical and chemical properties in this zone appears to indicate that the flooding waters from Hecate channel had a larger influence between the narrows than those from Nootka sound. How-

ever the observations at the entrance to the inlet (station I) were made near the time of low water slack and those near Tasis narrows (station K) were made at the full strength of the flood tide from which it is evident that this influence would be exaggerated in the data, although it has been observed that the area of the cross section of Tsowwin narrows is approximately the same as that of Tasis narrows, the velocity of the tidal current through the letter is much less than through the former, and as has been shown, the greatest influence in the inlet was the tidal current from Nootka sound.

Since practically all the tidal flow occurred in the lower part of the epithalassal zone of Nootka sound, it appears likely that only the influence of the semi-daily tide flow would be felt between Tsowwin narrows and the head of the canal, and that most of the action of the daily tidal cycle would be blocked by the threshold.

Tlupana Inlet

Tlupana inlet is a compound fiard (Carter 1934) with three smaller inlets, Hisnet inlet, Nesuk bay, and Mautcha bay opening from it. The mouth is broad and deep, and the bottom rises from 270 metres in Hanna channel to 224 metres at Hisnet inlet where there is a small bar. Immediately beyond this the water deepens to 270 metres to the opening of Nesuk bay, past which it shoals rapidly to 170 metres at Mautcha bay and to the surface at Suchwoa bay.

The mountains are between 2000 and 3000 feet (600 to 900 metres) high, and precipitous to the bottom of the inlet, which is very uneven. At the head of each of the subsidiary inlets there is a small mountain stream dependent on a very small drainage area. These streams were at their lowest levels at the time of the investigation, as there was no snow on the coast mountains.

The observations were not all made on the same phase of the tide, and in the consideration of the data allowance must be made for anomalies that may be introduced from this cause.

Station	Time observed	Tide phase	Height (metres)
2234-A	13:23-13:40	Flood	3.08
2234-B	14:10-14:25	H. W. slack	3.11
2234-C	15:38-15:52	Ebb	2.80

The range of the tide was 1.61 metres in the fall from 3.11 to 1.50 metres in 5 hours 41 minutes between 14:04 and 19:45 o'clock.

PHYSICAL OBSERVATIONS

There was no sharp physical division in this inlet to distinguish between the surface and the deep sound waters as separate layers, the small amount of fresh water from the land being thoroughly mixed with the sea water in the subsidiary inlets. The surface was comparatively fresh (figure 5, B) and warm, (figure 5, A) grading through the upper 6 metres to the typical circulatory waters of the sound, except at the mouth of the inlet where an indistinct thermocline was evident at 3 metres depth. The surface zone may be considered as consisting of those waters that had a density less than 24.00, and a temperature above 10° C., and it was continuous from the head of the inlet into Nootka sound.

TABLE IV

Depth metres	Cl°/₀₀	Temp. °C.	Density in situ σ_t	Diss'd oxygen Mg. A/L	Diss'd oxygen % sat'n	pH	SiO_3 Mg. A/L Si×10³	PO_4 Mg. A/L P×10³
				Station A				
1	12.35	16.0	15.07	0.6110	104.1	8.3	5.9	0.70
2	13.47	15.8	17.64	.6465	121.7	8.5	5.9	0.63
4	17.17	11.1	23.68	.6965	123.0	8.4	10.8	1.18
6	17.56	10.1	24.41	.6753	120.0	8.3	13.8	1.28
10	17.73	9.2	24.79	.5685	99.4	8.2	18.4	1.49
20	17.97	8.5	25.23	.3300	57.0	7.9	30.1	1.96
30	18.06	8.3	25.41	.3275	56.3	7.9	35.4	2.03
50	18.16	8.1	25.55	.2696	46.2	7.9	40.8	2.12
100	18.20	8.0	25.63	.2575	44.1	7.8	44.1	2.12
200	18.23	7.9	25.69	.2008	29.1	7.8	45.8	2.40
				Station B				
1	12.41	17.2	15.90	0.5580	106.6	8.3	5.9	0.33
2	13.62	16.3	17.75	.6830	130.0	8.3	6.7	0.35
3	15.80	15.8	20.87	.6275	129.9	8.3	14.1	0.36
4	16.47	13.2	22.32	.7280	135.7	8.2	14.6	0.36
6	17.43	10.8	24.10	.6760	121.2	8.0	15.8	0.46
10	17.65	9.4	24.65	.6170	108.0	8.0	16.6	0.73
20	17.82	8.6	25.01	.3413	59.0	7.9	30.1	0.74
30	17.90	8.3	25.16	.3370	57.8	7.9	36.3	1.04
50	18.06	8.2	25.41	.2682	46.0	7.8	45.0	1.19
200	18.23	7.9	25.69	.2642	42.2	7.7	52.9	2.42
				Station C				
1	11.86	17.3	15.12	0.6430	120.0	8.5	6.2	0.40
2	15.56	15.1	20.69	.7680	146.8	8.5	5.9	0.56
4	17.33	11.7	23.81	.7190	131.3	8.5	14.2	0.78
6	17.46	10.2	24.24	.6680	120.9	8.4	15.1	0.81
10	17.57	9.45	24.53	.6325	110.8	8.3	28.7	0.85
20	17.87	8.4	25.11	.2923	50.3	7.8	37.9	1.79
30	18.01	8.25	25.32	.2615	44.9	7.8	37.9	2.09
50	18.13	8.2	25.50	.2372	41.8	7.8	45.8	2.09
100	18.23	8.1	25.65	.1831	31.5	7.7	49.1	2.09
200	18.23	7.8	25.70	.0970	16.5	7.7	55.2	2.29

The influence of the rivers flowing to the inlet was minimal and the shallow surface layer formed by this fresh water was probably destroyed by the westerly winds which blow directly into the inlet at this season of the year.

Below this surface zone in the main inlet, the water was identical in character and continuous with the circulatory sound waters which flowed directly up the inlet on the flood tide and reciprocally on the ebb. The velocity which is small at all times was strongest at approximately 6 metres, decreasing towards the head of the inlet, and with depth to about 100 metres. Below this level there was no appreciable increase in the density and the whole zone was continuous with the waters at the entrance to the sound.

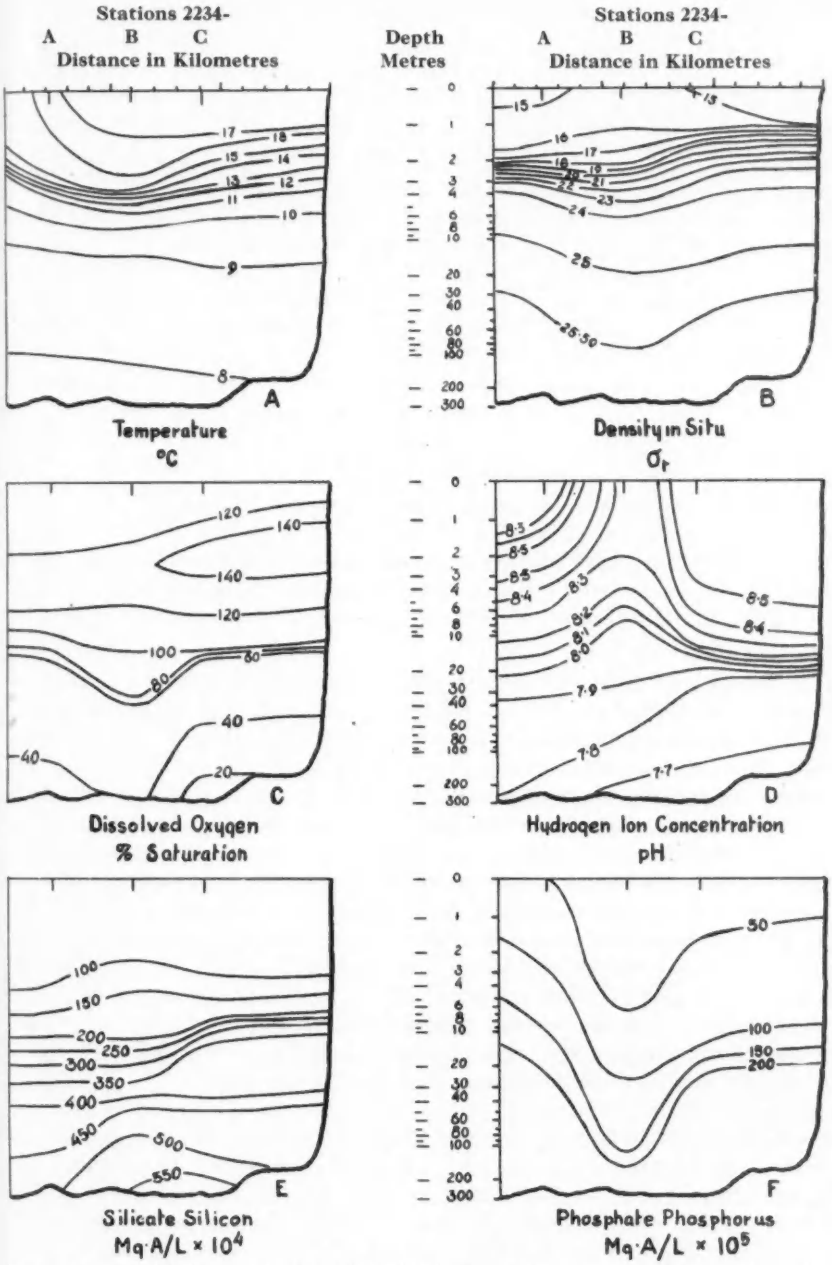

FIGURE 5. Graphical representation of the distribution of the physical and chemical properties of the waters in Tlupana inlet.

CHEMICAL OBSERVATIONS

The saturation with dissolved oxygen was very high (figure 5, C), particularly in the upper part of the inlet where it rose from 100 per cent at the surface to 140 per cent at 3 metres depth and fell again to 110 per cent at the boundary of this layer. The pH (figure 5, D) exhibited the greatest anomaly of any of the constituents, being high near the mouth and the head of the inlet, but comparatively low opposite Hisnet inlet where the values never rose above 8.3. This was probably due to the effect of the acid land waters, which are considerably protected from the wind in this subsidiary inlet, and any fresh water present had a greater opportunity to remain undisturbed in the epithalassium and probably had a greater effect on reaching the main inlet than that coming from the inlets to the southward, which are exposed to the full force of the wind. The concentration of dissolved silicates and phosphates (figure 5, E and F) was very low, although the former appeared to increase slightly in the region of the lowest pH, which tends to confirm the deduction regarding the influx of land water at this point. It would appear that phosphates were a limiting factor in plankton development as shown by Atkins (1926).

Between the head of the arm and Nesuk bay and below 170 metres depth there was a small body of water in which the saturation with dissolved oxygen was very low. This may indicate partial stagnation which possibly is a seasonal condition, occurring only when the density gradient is sufficient to preserve the stability of these waters against the infinitesimal circulatory effort present at this depth.

The chemical properties of the water, like the physical properties, indicated a definite homogeneity. The surface layers consisted of a small amount of fresh water thoroughly mixed with the sea water and showed indications of great photosynthetic activity and graded into the true coast waters without a thermocline or sharp change in the density gradient with the increasing depth.

There was an effect of the changing tidal phases during the progress of the observations that is noticeable in the level of the isometers of the various properties. Station B (figure 5) was observed at slack water and may be taken as the mean of the conditions during the observation of the inlet. At station A the flood tide waters are shown forcing the warmer surface waters back into the inlet so that they are about 1 metre deeper opposite Hisnet inlet than at the mouth. At station C, which was observed on the ebb tide, the effort of flow is evidently in the opposite direction; the surface layers tended to spread towards the mouth of the inlet and to become thinner, and the circulatory layers flowed in a reciprocal manner to the flood.

MUCHALAT INLET

Muchalat inlet is a true fiord (Carter 1934) as shown by the profile of the bottom (figure 6) which indicates the deepest channel from the mouth to the head of the inlet. The water at the entrance is deep, and shoals to a threshold at 120 metres depth at the east end of Gore island. The main part of the inlet is deeper than any other part of the sound. King passage south of Gore island is

so shallow that, in comparison to Williamson passage, its influence may be neglected.

The mountains are high and precipitous, ranging from 3000 to 4000 feet (900 to 1200 metres). There is no snow on these in midsummer, consequently the small coast streams were very low at the time of these observations. The Ahaminaquus river, which enters the inlet at the head of the main reach, drains a large basin which extends inland to the glaciers of the central range of Vancouver island. It is the largest stream flowing into any part of the sound, and even at this time of the year it carried a considerable volume.

The station at the entrance (H) was observed at the beginning of the ebb tide on July 25, and the other two stations (O and N) were observed during the corresponding tide of July 27. The range of these tides was the same and the weather was fine over the whole period, hence for the purposes of this report the observations may be taken as continuous.

Station	Date	Time observed	Height of tide (metres)
2235-H	July 25	15:35-15:50	3.05
2235-N	" 27	16:08-16:30	3.20
2235-O	" 27	17:23-17:46	2.68

The range of the tide was 1.83 metres in 5 hours 52 minutes on July 25 and 1.83 metres in 6 hours 17 minutes on July 27.

PHYSICAL OBSERVATIONS

There was a true epithalassium present in the main reach of the inlet which was clearly marked by a thermocline and a sharp rise in the density between 2 and 4 metres. The temperature gradient (figure 6, A) in this zone was not entirely regular nor was the thermocline at the same depth over the whole inlet. In general the temperature decreased from about 16° C. at the surface to 11° C. at the contact with the underlying zone. At the mouth, the gradient was regular and the thermocline was not distinct, but east of Gore island the surface was about one degree colder than the water between one and 2 metres depth, and the thermocline was quite sharp, particularly at station O, and clearly identified the surface zone.

The density (figure 6, B) rose slowly along the surface from the head to Nootka sound and with depth to 2.5 metres, where it increased suddenly to sound water magnitude except at the mouth where the rise was more gradual.

At the point of entry, the cold Ahaminaquus river water mixed with the warmer brackish water of similar density and sank, causing the epithalassium to be deeper at this point than in the middle of the inlet (Witte 1910 quoted by Sandstrom 1918) and the resultant brackish water had a tendency to spread out over the surface causing a definite flow towards the mouth of the inlet. There is very little strong wind at this season and, in their progress towards the mouth, the fresh and salt waters mixed by circulatory means only, so that there was a regular rise in density from the mouth of the river to Hanna channel.

The cold nature of the fresh water is explained by the steep gradient of the

TABLE V

Depth (metres)	Cl°/°°	Temp. °C.	Density in situ σ_t	Diss'd oxygen Mg. A/L	Diss'd oxygen % sat'n.	pH	SiO_3 Mg. A/L Si×10³	PO_4 Mg. A/L P×10³
			Station 2235-H					
1	10.31	16.0	13.26	0.5980	108.4	8.3	12.6	0.55
2	10.93	15.6	13.99	.6095	110.6	8.3	12.6	0.63
4	14.99	13.2	20.27	.6995	127.6	8.3	7.2	0.63
6	17.30	11.15	23.86	.7075	128.0	8.3	6.8	0.75
10	17.83	9.2	24.94	.4460	78.1	8.0	27.6	1.45
20	17.85	8.7	25.04	.3530	61.1	8.0	33.8	1.94
30	18.00	8.3	25.31	.3153	54.2	8.0	34.8	1.94
50	18.05	8.05	25.41	.2708	46.4	7.9	38.2	2.19
100	18.15	7.9	25.58	.2696	46.0	7.9	38.2	2.25
200	18.17	7.8	25.63	.1725	29.4			
			Station 2235-O					
1	6.08	15.9	7.46	0.5625	96.8	8.1	25.0	0.16
2	6.48	16.3	7.93	.5580	97.4	8.2	23.7	0.51
4	17.49	10.6	24.23	.6710	120.1	8.3	13.8	0.83
6	17.76	9.9	24.72	.6930	122.9	8.3	7.1	0.67
10	17.97	9.2	25.14	.5750	100.5	8.3	13.1	1.11
20	18.05	8.8	25.30	.2760	48.1	7.9	31.6	2.31
30	18.08	8.6	25.37	.2532	43.8	7.9	35.5	2.31
50	18.17	8.2	25.57	.2395	45.3	7.9	40.3	2.35
100	18.23	8.0	25.67	.2270	38.9	7.9	48.1	2.63
200	18.35	7.9	25.86	.0644	11.0	7.7	55.2	3.18
350	18.45	7.8	26.02	.0248	4.2	7.9	54.0	3.50
			Station 2235-N					
1	2.83	15.9	3.97			7.9	39.1	0.07
2	3.79	16.0	4.28	0.5750	96.5	8.0	36.8	0.39
4	16.63	11.6	22.85	.6710	121.2	8.3	21.0	0.71
6	17.54	9.65	24.46	.6890	121.1	8.3	14.5	0.52
10	17.75	8.8	24.89	.4260	73.9	8.0	18.2	1.16
20	17.97	8.4	25.25	.2316	39.8	7.9	34.2	1.90
30	18.06	8.3	25.39	.2316	39.8	7.9	38.4	1.90
50	18.09	8.15	25.45	.2138	36.6	7.8	43.9	1.96
100	18.15	8.1	25.55	.1660	28.5	7.8	45.8	2.17
200	18.34	7.9	25.84	.0666	11.4	7.7	54.1	2.27
350	18.34	7.8	25.86	.0260	4.4	7.65	56.3	2.62

river course, which causes a rapid flow, which in turn prevents the sun from warming the waters. The cold fresh water tends to keep the epithalassium at a lower temperature than would normally be expected; the flow is towards the mouth of the inlet and while the flood tide retards this movement, it was observed that the direction of the surface flow did not change.

The zone below the epithalassium was identical and evidently continuous with the circulatory waters at the entrance to Nootka sound. It was bounded by the isometers of density 22.50 to 25.50 and included the range of temperature 11° C. to 7.8° C. The tidal effort was transmitted through this zone on the

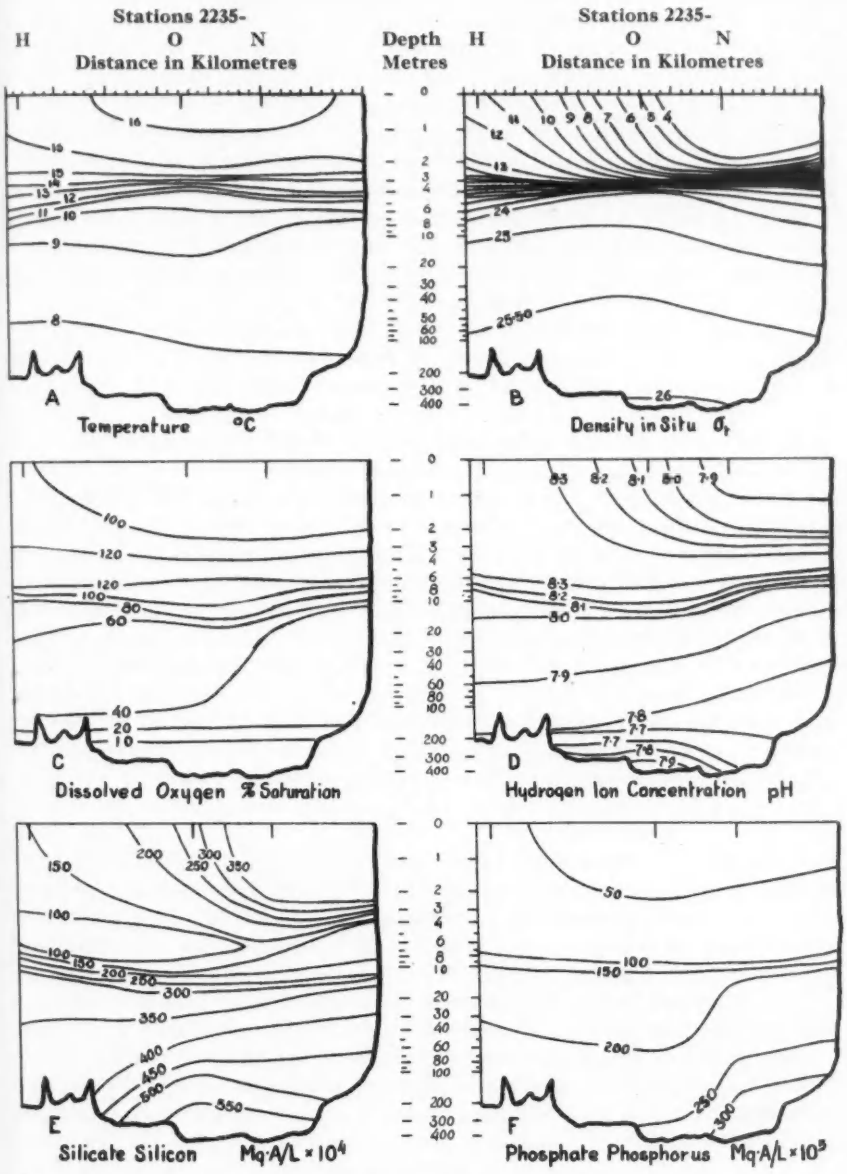

FIGURE 6. Graphical representation of the distribution of the physical and chemical properties of the waters in Muchalat inlet.

flood tide, the water flowing through Williamson passage where the maximum velocity was attained, diminishing rapidly in the larger space of the inlet. There was also a small flow through King passage which was not significant in comparison to that through the larger channel. The greatest effort was probably below the thermocline between 5 and 10 metres, diminishing with increasing depth to the level of the bar at 120 metres. The velocity was very small at this depth as is demonstrated by the distribution of the isometers of temperature and density, which show no tendency to drop below the level of the threshold in the main reach as in Tasis inlet. The tendency of the surface layers to flow westward was against this current, and east of Gore island the surface velocity in the direction of the flood was very small or non-existent, depending on the amplitude of the tide.

The run of the ebb tide is reciprocal to the flood and is similar in character throughout the circulatory zone, but the flow of the surface waters is additive and the maximum velocity is to be observed at the surface.

Below the circulatory zone, in the main basin of the inlet, the density rose to greater values than any in the sound, and it is evident that these waters do not regularly circulate over the threshold in Williamson passage, but it is noticeable that there was no sharp change in the density between this and the tidal zone which indicates that circulation was not entirely stopped. During the winter when the sound waters become colder and more dense than in the summer, there may be regular circulation in the lower levels which may reach a depth of 200 metres, below which the waters are of so much greater density than those in the sound, that total stagnation is probable.

Physically the waters were divided into four zones; the epithalassal, consisting of brackish water which had a current effort towards the mouth of the inlet; the zone of tidal circulation, which was made up of sound waters flowing in and out of the inlet with the tide; a layer in which circulation was irregular; and a body, below 200 metres, which is probably stagnant.

CHEMICAL OBSERVATIONS

The degree of saturation of the epithalassium with dissolved oxygen, increased regularly from less than 100 per cent at the surface to 120 per cent at the thermocline (figure 6, C). The lowest surface concentration was in the neighbourhood of the Ahaminaquus river, indicating that the water from this source was not saturated.

The characteristically acid nature of the drainage waters was very evident from the distribution of the pH (figure 6, D). The values increased with depth from the surface to 5 metres and with distance from the river towards the mouth of the inlet in a similar manner to the density, and the maximum value was in the thermocline in the region of the greatest concentration of dissolved oxygen.

The fresh waters carried a very high concentration of dissolved silicates (figure 6, E) which became distributed throughout the surface layers, the concentration decreased away from the river and with depth, the minimum value, approaching depletion, being reached in the thermocline. The distribution of the dissolved phosphates was opposite (figure 6, F), the concentration being

very low at the surface and increasing regularly with the distance from the river and with depth.

In the zone of tidal circulation the saturation of the water with dissolved oxygen was 120 per cent at the contact with the epithalassium. It fell rapidly from this value to 42 per cent at 100 metres at the entrance to the inlet, and at 18 metres opposite the river. The distribution of dissolved silicates and phosphates was very similar, passing through the ranges associated with the corresponding oxygen saturation in the sound.

The isometers of 100 per cent and 42 per cent of dissolved oxygen mark the limits of regular tidal circulation, the waters enclosed being continuous in every respect with those observed off Yuquot point. The fact that the isometers of the various characters are very nearly horizontal shows that the tidal circulation was gentle, diminishing in velocity towards the head of the inlet, and that the main force of the tide was below 5 metres and decreased with depth. The distance between isometers at any place may be taken as a direct relative measure of the tide force at that point.

In the uppermost part of the circulatory zone, just below the thermocline and the sharpest gradient of the density, the maximum concentration of oxygen occurred. This is an indication of strong photosynthetic activity in this narrow zone between 3 and 6 metres, which is given further support by the occurrence of the maximum pH values and the minimum concentration of dissolved silicates at this level. The concentration of dissolved phosphates did not reach normal values above 8 metres, although below 5 metres there is no appreciable dilution by the phosphate deficient fresh waters, from which it must be inferred that the phosphates in this zone between 5 and 8 metres were being used up, presumably by phytoplankton.

Below the level of 42 per cent the oxygen saturation fell slowly to 4 per cent at the bottom. At 200 metres depth the value is 10 per cent which marked the probable upper limit of complete stagnation, and this isometer, unlike those above it is horizontal, indicating that the tidal circulation had no effect whatever at this level.

Between the circulatory and the stagnant zones there was a body of water whose physical properties were horizontally continuous with those in the sound but whose chemical properties indicated that they had not been in circulation for some time. It is probable that during the winter months, when the tidal waters are colder and more dense and the vertical stability is at a minimum, circulation occurs to a greater depth than is apparent in these observations. The depth to which circulation may be observed evidently varies inversely as the temperature of the circulating waters, since the deeper waters, judging by the oxygen content, have been stagnant longer than the upper layers.

In the permanently stagnant zone the pH increased from 7.7 at 200 metres to 7.85 at the bottom and the concentration of dissolved phosphates and silicates rose to high values similar to those noticed in the deep waters of other parts of the sound.

A bottom sample taken at station N consisted of a green clay overlaid with a black organic ooze, smelling strongly of hydrogen sulphide, and showing no signs of living organisms.

CONCLUSIONS

The summer conditions in this area imply a vertical stability which divides the waters into well defined zones, the surface or epithalassium which is characteristic of its locality, and, below this, the circulatory zone in which the direct effort of tidal flow is located. In the fiords there is a zone in which circulation is intermittent depending on vertical stability, and below this a zone of total stagnation.

The epithalassium is subject to the winds and the boundary between it and the circulatory waters may easily be destroyed. This is particularly true in the sea outside the sound where there is no evidence of a thermocline separating the two zones.

The epithalassium in the inlets is warm and shows indications of dilution with land drainage waters. This dilution effect becomes less evident towards the open sea and the identity of these sound waters is lost in the surface coast waters about 20 kilometres south of Yuquot point.

The main effort of tidal flow is in the upper part of the circulatory zone between 3 and 15 metres and the velocity decreases with increasing depth. The thresholds near the entrances to Tasis and Muchalat inlets block the efforts of tidal circulation so that the waters below their levels tend to become stagnant. This is evident from the density and the dissolved oxygen content and the presence of hydrogen sulphide in the deep basins of these inlets. At this season of the year the vertical stability tends to limit the action of the tidal currents, so that there is little or no effort below 100 metres depth. In some of the inlets, notably Tasis and Muchalat, the physical properties of the water below the level of the thresholds show that some of the water is continuous with that in the sound, but the chemical properties indicate that it has not been in circulation for some time. It is probable that in the winter season when stability reaches a minimum, there is little or no resistance to a vertical component in the circulation, and part at least of the deep zones which are stagnant in the summer becomes circulatory, preventing the extreme effects of stagnation which are found below 100 metres in Tasis and 200 metres in Muchalat inlet, and the upper level of stagnation probably varies in depth inversely as the temperature of the circulating water.

There is evidence of two separate cycles of reciprocal circulation near the entrance of the sound. The surface layers appear to have a semi-daily cycle harmonious with the rise and fall of the tides, and the waters below 8 metres appear to ebb and flow only once during the day. No explanation is at present offered for this phenomenon, as it is being investigated separately and may afford some explanation for the difference in time between the phase and flow of the tide.

The surface currents of the flood tides are somewhat weaker in the inlets than those of the ebb. This results from the continuous flow of fresh water towards the sea from the head of each inlet, which is most pronounced in Muchalat inlet and negligible in Tlupana inlet.

There is evidence of upwelling of the deep Pacific waters about 20 kilometres southwesterly from Yuquot point, which is shown by the low temperature and oxygen content of the water at 100 metres depth.

The greatest anomalies in the chemical properties, such as supersaturation with dissolved oxygen, high pH, and low concentrations of dissolved silicates and phosphates, occur between 3 and 6 metres depth, indication that the maximum photosynthetic activity probably occurs in this zone.

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Studies on Salt Fish.
1. Bacteria Associated with the Reddening of Salt Fish

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ABSTRACT

A study has been made of thirty strains of red halophilic organisms from reddened fish, salt and the timbers of salt fish plants. Eleven were cocci and belong to the species *Sarcina litoralis* and *Sarcina sp.* of Lochhead. The rod forms are of five types. Three resemble *Bact. trapanicum* Petter, *Serratia salinaria* and *Serratia cutirubra* Lochhead. The others do not resemble any of the described species. Of thirty samples of salt, reddening organisms were found in twenty, seven of which were mined or evaporated salts.

During the past few years the amount of salted fish put up in the Maritime Provinces has decreased considerably. This is due largely to economic conditions and factors beyond the control of the industry. Any condition which might cause further loss in such an industry is important, and the loss caused by the reddening of salted fish has been a problem for many years. Reddening is caused by the growth of a group of halophilic bacteria. As these bacteria multiply the fish become covered with a layer of organisms varying in colour from pinkish to dark red. This reddening is a factor in the loss of some markets, as fish from other countries may "stand up" better in some climates, and the increased cost of control measures makes competition impossible.

Reddening is known in all parts of Nova Scotia where fish are salted. It is also known in the southern part of Prince Edward Island, in the Magdalen islands and along the bay of Fundy shore of New Brunswick.

The loss caused by reddening is difficult to estimate. Many producers do not like to admit they have losses. Sometimes in very hot seasons the fish redden in the sheds. More often it is after the fish have gone to the wholesaler or retailer, or have been exported to warmer climates. The small losses of the fishermen, important as they are to him, form a considerable aggregate. Foreign losses, at least in some importing countries, have been given as from five to ten per cent.

In spite of the paucity of actual information, the number of requests for methods of dealing with the problem, and information from persons in close contact with the industry, indicated that an investigation was warranted. This study is, therefore, another of the many attempts to find a method of controlling the condition. This paper deals with some of the organisms concerned.

HISTORICAL

Apparently the reddening of brines has been known almost as long as the art of making salt. Baas-Becking (1931) quotes from the ancient Chinese treatise Peng-Tzao-Kan-Mu (about 2700 B.C.) that in the province of Yai-cheau to extract salt from sea water "An embankment is made and ditches to draw clear sea water. It is left a long time until the color becomes red".

Todd (1683) mentions a probable occurrence of red bacteria in a salt spring in Durham, England,—"Those who have boiled the Brine say, that it affords a great Quantity of Bay Salt, not so palatable, yet as useful as ordinary Salt is. It tinges all the Stones with a red Colour."

The first scientific study of reddening of salt fish was that of Farlow (1880). Since then the condition has been studied by many investigators. The older studies have been reviewed in detail by several authors (Kellerman (1914), Harrison and Kennedy (1922), Tattevin (1926-27)). The earlier investigators confined themselves to morphological studies which led to species differentiation on size and arrangement. From recent observations it is probable that natural variability of the organisms would account for most of these differences. Harrison and Kennedy (1922) recognised this in respect to *Serratia salinaria*. Petrova (1932) believes that all of the coccus forms described are identical and suggests the name *M. roseus halophilus* or *salinarius*. More recently Lochhead (1934) described two species of red cocci and two new rod forms from salted hides. Stather (1930) claims that the organisms he isolated from hides and grew at low salt concentrations are identical with those isolated by Lloyd (1929) as strict halophiles. This point is also stressed by Stuart et al. (1933), who claim that while the organisms resemble typical bacterial forms at high salt concentrations, if adapted to lower concentrations they resemble the higher bacteria. Stuart and Swenson (1934) have shown that the morphology and also physiological characteristics may vary with the salt concentration. Stuart (1935) has shown that with *Myxococcus rubescens* the morphology is influenced very decidedly by the kind of available organic nutrient in the medium, and that it may vary from coccus forms, through definite rod forms to long filaments.

In the present investigation, being interested only in the behaviour of the organisms on a heavily salted medium, salt fish, no attempt was made to adapt the organisms to low salt concentrations.

METHODS

All early isolations were made on starch medium. This was prepared by mixing 175 gm. flour (wheat) and 100 to 110 gm. sodium chloride, then adding 500 ml. of fish broth, stirring to avoid lumps. The resulting paste was poured into Petri dishes and sterilized in the autoclave. A fairly hard matt surface is thus obtained. By using a thin layer and allowing the autoclave to cool slowly, bubbling and lumpy surfaces are avoided.

Fish broth was made by boiling 500 gm. of ground fish muscle (cod) in 1 litre of water. This was then strained through several layers of cheese cloth. The

finely divided protein was not removed. For agar, 200 gm. of sodium chloride and 20 gm. agar are added to a litre.

Later the milk and yeast extract agars of Lochhead (1934) were used. These are more easily prepared than starch media and are very satisfactory. Generally speaking, the rod forms grow better on milk agar than on yeast extract agar, and do not grow on the latter if starch is omitted. The coccus forms grow better on yeast extract and will grow without the starch.

Growth in liquid media is not very satisfactory and for all differential tests solid media were used.

Nitrate reduction was tested by adding 0.2 per cent potassium nitrate to either milk or yeast extract agar. For indol production one per cent tryptophane broth (Difco) was added to the agars. In testing for indol the Goré method was used. Starch hydrolysis was tested by flooding yeast agar plates with Lugol's iodine or testing milk agar to which soluble starch had been added. Hydrogen sulphide production was tested for by adding lead acetate to yeast or milk agar. A blackening of the growth was considered a positive test. For salt potato, potato slants were soaked over night in 20 per cent brine.

Gelatin was made by sterilizing 300 gm. of gelatin in 1 litre of fish broth by intermittent sterilization. This was added to 200 gm. of sterile salt and the mixture tubed.

Agar media were used for hydrogen ion concentration tests. These were tested before sterilizing with the quinhydrone and glass electrodes. They were checked after sterilization with the quinhydrone electrode.

Incubation was carried out at 37°C., except in case of gelatin, which was incubated at 25°C.

Staining

For microscopical examination the organisms were suspended in 20 to 30 per cent brine. Smears were allowed to dry in air. The most satisfactory fixative was absolute methyl alcohol. This was allowed to remain on from 3 to 5 minutes and renewed as needed. As most stains are precipitated by salt, the stain was added directly to the alcohol on the slide. This seems to prevent the small particles of stain precipitating and interfering with the examination.

The one per cent aqueous solution of basic fuchsin used by Lochhead (1934) gives excellent results. Giemsa's stain applied for 20 to 30 minutes also gives good results. To our mind the best method is to apply the concentrated stain directly to the alcohol on the slide, which has been renewed if necessary.

For morphological examination the organisms were suspended in 20 per cent brine and examined unstained.

ORGANISMS ISOLATED

In the present study isolations have been made from salt fish, various salts and a few from salting establishments. These have been made over a period of three years. The isolations include eleven strains of coccus forms and nineteen rod forms.

Comparisons have been made with cultures received from other investigators. Grateful acknowledgment is made to Prof. A. J. Kluyver for Petter's strains of *Bact. halobium* and *Bact. trapanicum*; to Dr. A. G. Lochhead for cultures of *Sarcina litoralis*, *Sarcina sp.*, *Serratia salinaria* and *Serratia cutirubra*; and to Dr. L. S. Stuart for cultures of *Myxococcus rubescens*.

Coccus Forms

The red halophilic cocci isolated fall into two groups; eight strains resemble the form described by Lochhead as *Sarcina litoralis*, the others his *Sarcina sp.*

Sarcina (Micrococcus) litoralis

Strains were isolated from reddened fish, Turks Island and Cadiz salt.

It is difficult to say whether the organism is a *Micrococcus* or a *Sarcina*. We prefer the former classification as in most cases sarcinal forms were not obtained. The strain received from Lochhead does show mostly sarcinal forms on yeast extract agar. Our strains resemble it so closely that they are probably identical. In our experience pairs and tetrads were the most frequent arrangement and the higher the salt concentration the greater the tendency to form tetrads.

The organism grows at salt concentrations of 10 to 35 per cent, with slight growth at these limits (throughout the paper per cent refers to the number of grams of salt added to 100 ml. of medium). The optimum growth is at 20 to 25 per cent salt. Growth was obtained over a pH range of 5.5 to 9.0, with best growth at about 6.5.

Indol is produced. Dextrose and lactose are not fermented.

Sarcina sp. Lochhead

Three strains from red fish and Torrevieja salt correspond to the *Sarcina sp.* of Lochhead (Culture 63-R1).

This organism occurs mostly as a sarcina, less frequently in fours and pairs. On yeast extract the growth is lighter in colour than *Sarcina litoralis*, more of a rose red, which deepens with age. The growth is duller and slightly roughened. On milk agar the colour is lighter than on yeast extract, and the growth not as rough.

It is similar to *Sarcina litoralis* in salt, pH and temperature range. Gelatin is not liquefied. Starch is not hydrolysed. Nitrates are not reduced. Indol and hydrogen sulphide are produced. Dextrose and lactose are not fermented. Growth on salt potato slight or none at all; slightly better on fish broth salt potato. It is an obligate aerobe.

This organism differs from *Sarcina litoralis* in that the sarcinal form is more common. Its colour is lighter and the appearance on agar is rougher. We have not noticed the difference in salt range noted by Lochhead.

Rod Forms

The rod forms have been placed in Part II of the genus *Serratia* (Bergey 1934). Recently, it has been suggested (Breed 1936) that the species in Part II

of this genus be placed in a new genus, probably *Proactinomyces* (Jensen 1931, 1932). It is very probable that the non-motile species described in this paper could be placed in this new genus.

Bacterium trapanicum Petter

The most common of the rod forms resembles *Bact. trapanicum* Petter (1931). It was isolated from reddened fish, Port Said, Cagliari, Malagash, Liverpool evaporated and Turks Island salts.

On milk agar it is a rod, 0.5 to 0.8 x 1 to 3μ, with many short, almost coccoid forms. Older cultures show longer forms, oval and disintegrated cells (symplasm). On yeast extract agar there are round forms, short rods and irregular forms. The organism is motile.

Milk agar. Good growth, smooth, raised, glistening, slightly viscid, entire edges. Orange red. No proteolysis.

Yeast extract agar. Good growth, similar to milk agar. Colour more orange.

Starch medium. Duller growth, sinking into medium.

Salt potato. Good growth, smooth, glistening, orange red to brownish orange.

Gelatin not liquefied. Indol not formed. Hydrogen sulphide produced. Nitrates reduced to nitrites. Starch not hydrolysed. Slight acid production in dextrose.

Salt range. 10 to 35 per cent. Optimum, 20 per cent, but in most cases excellent growth at 15 and 25 per cent.

pH range. 5.5 to 8.5, optimum about 6.5.

This organism differs from the other rods isolated in that growth is equally abundant on both milk agar and yeast extract agar.

It resembles *Bact. trapanicum* Petter in its reactions and morphology, although the organism received from Prof. Kluyver is of a brighter red.

Serratia sp. a.

This organism was isolated from Turks Island and Malagash salts and from timbers in a salt fish plant.

On milk agar, young cultures consist of round or slightly oval forms, 1.0 to 1.5μ in diameter. In older cultures these give way to short rods, 0.8 x 1.2 to 2.0μ. On yeast extract agar there are a few rod forms but the majority are round, 0.8 to 1.0μ in diameter. It is non-motile.

Milk agar. Good growth, raised, smooth, iridescent, butyrous, pink becoming deeper with age. No proteolysis.

Yeast extract agar. Good growth, convex, smooth, glistening. In most cases colourless, sometimes shows streaks of red, also clear areas which look like bubbles of gas in the growth, giving it a grumose appearance.

Starch medium and fish broth agar. Good growth, similar to milk agar.

Salt potato. Slow growth, brownish yellow.

Gelatin not liquefied. Starch not hydrolysed. Indol and hydrogen sulphide produced. Nitrates reduced to nitrites.

Salt range. 10 to 35 per cent. Optimum, 15 to 20 per cent.

pH range. 5.5 to 9.0, optimum about 6.5.

Temperature range. 20° to 53°C. Optimum, 37 to 42°C.

Serratia sp. b.

One strain was isolated from Turks Island salt.

On milk agar the organisms are short rods, 0.8 x 1.5 to 2.0μ. On starch media they are slightly longer. On yeast extract agar there are some rod forms but more ovals; in older cultures irregular forms predominate.

Milk agar. Growth convex, entire, smooth, glistening, soft, butyrous, rose pink. Decided zone of proteolysis, most pronounced at optimum temperature.

Yeast extract and fish broth agar. Growth similar to milk agar. Zone of clearing around growth on fish broth agar.

Starch medium. Growth flatter and slightly rougher.

Salt potato. No growth.

Gelatin liquefied. Starch not hydrolysed. Indol and hydrogen sulphide are produced. Nitrates reduced to nitrites.

Salt range. 10 to 35 per cent. Excellent growth from 15 to 25 per cent.

pH range. 5.5 to 9.0, with good growth from 6.5 to 8.2.

Temperature range. 25 to 53°C. Optimum, 42°C.

The organism is strictly aerobic.

Serratia sp. a and *b* do not fit any of the descriptions which have come to our attention. We believe them to be new species.

Serratia salinaria

This organism was isolated from several samples of salt fish and from Torrevieja salt. It is identical with the strain of *Serratia salinaria* received from Lochhead and described by him (1934).

It has been noticed that although the organism is proteolytic when first isolated, this property is gradually lost in many cases. There is good growth on salt potato. Indol is produced. The pH range is from 5.5 to 8.0, with the optimum at about 6.8.

Serratia cutirubra Lochhead

This organism was isolated from reddened fish, Torrevieja, Port Said and Cagliari salts.

We believe this organism to be the same as that described by Lochhead (1934) as *Serratia cutirubra* n.sp. Old cultures change from a red to a mauve or reddish mauve, which seems to be a definite characteristic. Although Lochhead reports that indol is not produced, slight indol production has been obtained from all our strains.

EXAMINATION OF SALTS

Thirty samples of salt have been examined for the presence of red halophilic bacteria. The salt was seeded onto plates of media containing about 20 per cent salt. Twenty of the samples were positive for red organisms of one type or another. Some showed very few organisms but no attempt was made to be quantitative.

All the samples were obtained from fish plants or dealers in Nova Scotia and represent the kinds of salt most commonly used in fish salting. They included Malagash and German mined; Malagash and Liverpool evaporated; and solar salts from Turks Island, Torrevieja, Cadiz, Cagliari, Sfax and Port Said.

Due to the heat applied during evaporation, evaporated salts must be sterile, or at least free of red organisms, at the time of preparation. The large percentage of contaminated samples found in this class shows that salts may become infected

TABLE I. The occurrence of reddening organisms in salts

Type of salt	Number of samples		
	Examined	Positive	Negative
Solar	13	13	0
Mined	9	1	8
Evaporated	8	6	2
	30	20	10

in storage. It is apparent therefore that because a salt is evaporated or mined is no assurance that it does not contain red organisms. Stuart et al. (1933) found 97 per cent of their solar and 64 per cent of their open pan evaporated salt samples contained red organisms.

SUMMARY

A study has been made of thirty red halophilic organisms from reddened fish, salts and salt fish plants. Eleven of these were cocci and identified as belonging to the species *Sarcina litoralis* and *Sarcina sp.* of Lochhead. The rod forms were divided into five types. Three were similar to *Bact. trapanicum* Petter, *Serratia salinaria* and *Serratia cutirubra* Lochhead. The other two do not resemble any of the described species and are believed to be new species.

Thirty samples of salt were examined for the presence of reddening organisms, which were found in twenty. Seven of the positive samples were mined or evaporated salts.

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The Measurement of Spoilage in Fish

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ABSTRACT

The increase in volatile nitrogenous bases in codfish muscle between the pre-rigor period and the first appearance of odour is approximately 6 mg. per 100 grams of tissue, and is due almost entirely to the action of bacteria. It can be used to follow the course of spoilage only if the original value of the fish in question is known, as the range in variation of the original values is as great as the increase to the appearance of odour.

A method for the rapid determination of "trimethylamine" in cod muscle has been devised. Its increase parallels the increase in bacterial population. Odours always appear at approximately the same level of "trimethylamine". The increase resulting from autolysis is negligible. The increase during the development of spoilage is fifteen to twenty times the original value. Spoilage can be followed in fish preserved with borates as well as in untreated fish.

In the production of perishable foods such as fish and meats, an accurate method for the detection of incipient spoilage is needed, not only to make it possible to prevent slightly tainted foods reaching the consumer but for the intelligent production and control of these food products.

The literature concerning tests for freshness in meats and fish has been reviewed by Weaver (1927), Fellers, Hunter and Korff (1929) and Boury and Schvinte (1935). Available methods may be divided into five classes, organoleptic tests, bacterial counts, reduction reactions, physical methods and the measurement of the end products or by-products of bacterial metabolism.

Tests dependent primarily on the senses of smell, taste, sight or touch are the only ones that have been applied generally in commercial practice. They are useful in that they can be applied rapidly, and in that they provide some information as to the quality of the fish. They are faulty in that they are at best only qualitative and indicate very roughly the degree of spoilage that may have taken place. They are dependent on the sharpness of the senses of the observer, and not only are the results of different examiners at variance, but the same examiner may differ from day to day and from sample to sample.

Bacterial counts as a measure of fish spoilage are open to two serious objections. In the first place the method is too time-consuming for practical purposes and secondly the determination of bacterial numbers in semi-solid material such as fish muscle is probably not very accurate.

Reduction reactions such as the reduction of nitrates and methylene blue have been used for the detection of incipient spoilage in meat and fish, but the

disagreement in the literature would indicate that these tests are of doubtful value (Tillmans and Otto 1924; Arbenz 1925; Weaver 1927).

Attempts to measure spoilage in fish by physical means such as the measurement of the elastic properties of the flesh have been made by Tauti (1931) and Yamamura (1932). The results seem to indicate some degree of success with this method. It is only necessary to point to such factors as spawning, bruising while in the trawls, and pressure while in the pens aboard the vessels, all of which influence the texture of the flesh, to show that this method is probably not applicable to cod, haddock and such "ground" fish.

Chemical tests measure either the rate of bacterial metabolism or autolytic breakdown or both. Changes in the content of non-protein, soluble protein, and amino nitrogen have been used in studies of fish decomposition, but because of lack of sensitivity have not proven satisfactory. Usually some end product of bacterial metabolism is chosen as a measure of spoilage.

Stansby and Lemon (1933) published a method for the detection and measurement of incipient spoilage in fish muscle which is sufficiently rapid to be of practical value. Because this method has received a considerable amount of attention, it will be discussed in more detail. The method is dependent on the supposition that the opening of the protein molecule and the production of compounds of lower molecular weight must precede the production of end products such as ammonia, amines or indole. These so-called primary changes are determined by the measurement of the buffering capacity of aqueous extracts of the muscle.

The premise on which the method is based is faulty in that the "secondary products," especially amines, may not be derived from proteins but from the non-protein nitrogenous constituents of the muscle tissue. The results show a gradual decrease in buffering capacity of the extracts. This is contrary to expectations, because the opening up of the proteins, and the resulting increase in the number of free amino and carboxyl groups should produce a greater buffering effect. Finally, Nickerson and Proctor (1935), using samples of sterile and contaminated muscle from the same fish, obtained parallel reductions in buffering capacity in both samples. They believe this to be due to the fish having been frozen. Finn (1934) has shown that fish muscle proteins are denatured by freezing. One may conclude, therefore, that the effect obtained by Stansby and Lemon is due not to bacteria, but possibly to the denaturation of the muscle proteins.

Weaver (1927) used hydrogen sulphide production to detect spoilage in meat. Budagjan (1932) also recommends this method, but warns that negative tests should be confirmed by other methods, as decomposition does not always result in hydrogen sulphide production. Indol was used by Clough (1922) as a check on organoleptic tests in spoilage of salmon. Dauphinee (unpub.) found that indol was produced very rapidly in haddock after 60 hours at room temperature, the curve being very similar in shape to that for volatile bases.

Boury and Schvinte (1935) made an intensive study of the various methods of detecting incipient spoilage in fish and concluded that the best method was the determination of volatile nitrogen, amine or ammoniacal. Similar conclusions have been reached by others (Tillmans and Otto 1924; Glassman and Rochwarger

1929; Kimura and Kumakura 1934; Lüche and Gerdel 1935; Tanikawa 1935). It is generally agreed that the volatile bases increase early enough to be a measure of incipient spoilage, and the practical application of this principle depends on the development of a method which is rapid and simple enough for routine procedure.

VOLATILE BASES AS A MEASURE OF FISH SPOILAGE

The utilization of the concentration of volatile nitrogenous bases of fish flesh as a measure of its freshness is dependent on at least five conditions,—(1) the analytical procedure should be adequate both as to accuracy and speed, (2) there should be a definite relationship between increases in volatile bases and bacterial population, (3) odours suggestive of spoilage should appear in different fish after the same increase in volatile bases, (4) autolysis must play only a minor part in their production, and (5) the original values for volatile bases must be sufficiently low or sufficiently constant to make significant the increases resulting from bacterial action.

Analytical Methods

Former work based on aeration or distillation methods is for the most part open to serious objection, in that either aeration or distillation was used to recover the volatile bases. It is impossible to aerate quantitatively within reasonable time all the bases from the tissues. Either prolonged aeration or distillation may produce ammonia due to the action of alkalis on the tissues. The method of Parnas and Mozolowski (1927) was used in our experiments because (1) the alkali, sodium borate, is very mildly alkaline, pH 9.5, (2) the bases are distilled over at a temperature always below room temperature, (3) the length of the distillation period is not sufficiently great to hydrolyse alkali-producing substances and (4) under reduced pressure all the volatile bases can be recovered.

The original apparatus of Parnas and Mozolowski was used in our earlier experiments, but several modifications suggested themselves, and the apparatus finally used is shown in figure 1. This apparatus has several advantages over the original in that it can be all glass, the shape of the distillation flask facilitates cleaning, and the manipulation of the receiving flask is much easier. The distillation flasks are made from pyrex tubing of 22 mm. internal diameter and contain approximately 100 ml. to the neck. A notch is filed in the plug of stopcock 2 to enable better control of the steam. The condenser is of 7 mm. pyrex tubing and is 24 mm. in length. The receiver was made by cutting the neck from a 50 ml. pyrex Erlenmeyer flask, fusing on a piece of pyrex tubing at a slight angle, and grinding in a stopper as shown in the figure.

For the determination of volatile bases, an excess of standard acid approximately seventieth normal plus the methyl red methylene blue indicator of Wesslow (Cole) is placed in the receiving flask and the flask is turned to immerse the finely drawn tip of the condenser in the standard acid. Two grams of tissue plus 10 ml. saturated sodium borate are placed in the distilling flask. Stopcocks 1 and 2 are closed, stopcock 3 is opened and the apparatus is evacuated by means

of a water pump. Stopcock 2 is opened very slightly and steam is passed in at a very slow rate. There is a tendency towards frothing for a short time but if the steam is passed in slowly enough this is not bothersome. When the frothing subsides the steam is admitted at such a rate that approximately 15 ml. distillate is collected in 30 minutes, during which time all volatile bases are distilled over. With smaller samples of tissue distillation time is reduced considerably, but in rapidly spoiling tissue, smaller samples do not give consistent results. Tissue was ground with sand, and while frozen with solid CO_2, without materially speeding up the distillation. Therefore these procedures were omitted and approximately 50 gm. were ground in a mortar with pestle to fit, after which 2 gm. samples were weighed out. The slow rate of distillation is due apparently to the tendency of the tissue to build up to considerable thickness on the sides of the distillation flask and the tube through which the steam is admitted. Complete recovery of volatile bases was never accomplished either with the original apparatus or with the modified form in the time stated by Parnas and Mozolowski. At the end of the

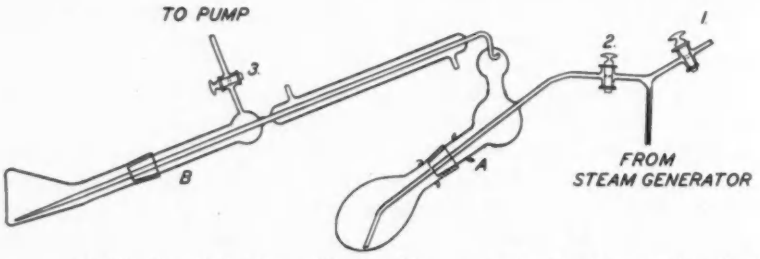

FIGURE 1. Modification of the Parnas-Mozolowski apparatus for the determination of volatile bases in tissue. Apparatus clamped at A and B.

distillation period the receiver is inverted, stopcock 3 is closed and stopcock 1 is opened, admitting air to the apparatus. The receiver is removed and the excess acid is back titrated with standard alkali, approximately seventieth normal.

RELATION TO BACTERIAL COUNTS

Because of the difficulty of counting bacteria accurately in the tissue itself the rise in the bacterial population and the increase in volatile bases were followed in muscle press juice. No particular pains were taken to prevent contamination of the press juice. It contained therefore bacteria from outside sources as well as the original fish flora. The press juice was divided into three portions, which were stored at 20° C., 10° C., and 0.5° C. respectively. At intervals samples were removed for bacterial counts and for the determination of volatile bases. The rise in bacterial numbers in comparison with the rise in volatile bases is shown in figure 2.

The shapes of the corresponding curves are very similar and there is reasonably good agreement in the actual percentage increase in each case. It was concluded, therefore, that, in press juice at least, the rise in volatile bases is a fairly accurate measure of the rise in bacterial population.

Relation to Spoilage Odours

The course of spoilage of some hundred fish, as shown by the rise in volatile bases, has been followed, and careful note has been made as to the volatile base level at which odours suggestive of spoilage put in an appearance. The point at which odours first appear could not be determined accurately, as examiners disagreed as to when putrefactive odours could be detected, but the results of these experiments indicate that spoilage odours appear after an increase of approximately 4 to 6 mg. volatile basic nitrogen per 100 gm. tissue, while the odours are very definite after an increase of about 12 mg. volatile basic nitrogen per 100 gm. tissue. It is felt that the agreement between the rise in volatile bases and the appearance of odours is as good as can be expected.

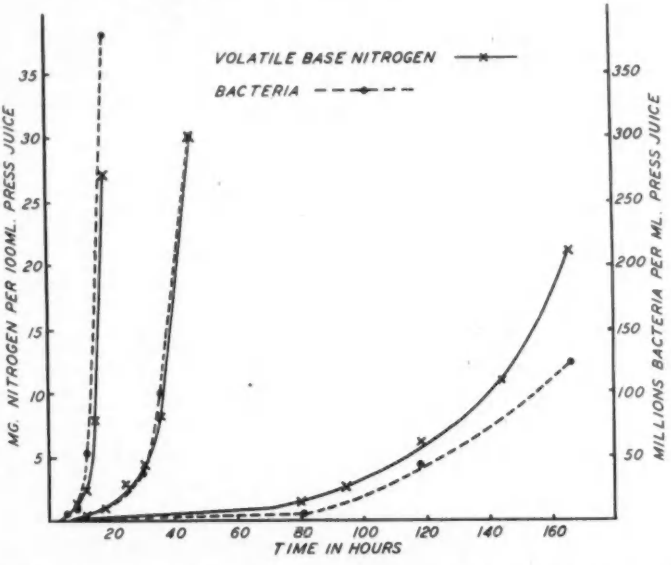

Figure 2. The increase of volatile bases and the rise in bacterial population in cod muscle press juice.

It will be recalled that the practical fish handler utilizes the appearance of the slime and the gills as a guide in estimating the quality of the fish. Further correlation between organoleptic tests and the rise of volatile bases is shown in table I.

The very rapid rise in the volatile bases in gill tissue agrees with the finding of the trade as to the comparative rate of spoilage in gill and in muscle. The results show also that while fresh gill tissue does indicate fresh muscle, gills may be definitely decomposed before the muscle tissue has undergone definite deterioration. At the end of approximately 20 hours gills of all fish were spoiled while muscle tissue of all was perfectly fresh, as determined by absence of odour and by the level of volatile bases.

TABLE I. The increase in total volatile basic nitrogen in the gills, peritoneum and flesh of cod at 20° C.

Fish no.	History	Tissue analyzed	Storage time (hours)	Volatile basic nitrogen (mg./100 gm.)
1	12 hours on ice.....	Peritoneum	0	12.0
			36	28.1
2	Brought alive to laboratory	Gills	0	4.5
			22.8	32.5
			48.25	52.2
		Muscle	0	7.2
			20.8	7.3
			49.8	13.1
3	" " " "	Gills	0	4.0
			21.1	20.0
			46.7	62.1
		Muscle	0	8.0
			18.5	8.4
			48.5	9.7
4	" " " "	Gills	0	3.9
			23.9	27.6
			45.9	57.6
		Muscle	0	8.0
			18.25	8.4
			47.75	12.1
5	" " " "	Gills	0	3.6
			22.6	32.7
			45.5	66.2
		Muscle	0	8.1
			23.25	7.9
			47.6	11.6

EFFECT OF AUTOLYSIS

The production of volatile bases by autolytic enzymes was followed by the incubation of sterile tissue. Sterile tissue is so difficult to obtain that considerable controversy has arisen as to whether or not living muscle is sterile. A number of sterile pieces of tissue, sufficient to serve our purpose, was obtained in the following manner. Codfish were brought to the laboratory alive. The whole of the surface slime was removed by scrubbing with 10% NaCl, 10% Na_2CO_3 solution. The fish were wiped dry and immersed 3 minutes in 1.5% formalin. The skin of one side was further scrubbed with iodine followed by alcohol. The skin from this whole side was peeled off with sterile pliers and pieces of tissue from the centre of the exposed area were removed, using aseptic precautions. These were incubated at 0°C. for 21 days. Sterility was tested by plating samples when analyzed and the results from those showing growth were discarded.

The original compared with the final values for the volatile basic nitrogen in mg. for 100 gm. of muscle in the case of four different fish were 9.3:13.8, 9.3:14.3, 8.9:13.8 and 11.5:16.1.

This shows that in the time of the experiment the rise in volatile base is very small, being never more than 5 mg. %. In table II is shown the rate of production of volatile base typical of fish handled in the present commercial manner.

TABLE II. The increase in total volatile basic nitrogen in commercial cod fillets stored at 0° C. Values expressed as mgs. nitrogen per 100 gm. tissue

Storage (days)	Fish number						
	1	2	3	4	5	6	7
0	8.3	9.4	9.9	8.9	7.9	7.1	6.2
8	9.9	9.1	9.7	9.0	7.5	8.3	6.5
9	10.3	10.0	9.8	....	9.2	9.5	7.6
10	12.8	12.6	12.5	9.1	12.4	13.5	10.1
11	15.6	12.4	11.0	13.0	21.1	22.2	13.8
12	18.4	19.8	14.9	....	....	....	....
14	32.6	....	33.7	31.5	....	....	....

In 14 days or less the increase in volatile base is from 7 to 24 mg. %. While there is a rise in volatile bases due to autolysis it is negligible in comparison to that due to bacteria.

ORIGINAL VALUES

Since the volatile bases increase with the rise in bacterial population, since odours suggestive of spoilage appear at approximately the same level of volatile bases, and since autolysis plays a very small part in the production of volatile bases, the course of spoilage of any fish can be followed with reasonable accuracy. The rise from the value of fish as removed from the water to that of a fish approaching spoilage is approximately 6 mg. volatile basic nitrogen per 100 gm. tissue. Before the method can be applied to the examination of fish in general, it must be ascertained if the variation in the original value is sufficiently small to make this rise of 6 mg. per 100 g. tissue significant. The original values for the volatile basic nitrogen have been determined in the course of our experiments over a period of approximately a year. Values have varied from 5 to 12 mg. per 100 gm. tissue; that is the variation from fish to fish may be as great as the increase from the values as caught to that of the first appearance of spoilage odours. This is in confirmation of the findings of Reay (1935).

DISCUSSION

From the preceding work it is obvious that the concentration of volatile bases cannot be used to detect the earlier stages of fish spoilage unless the original value for the fish under examination is known. But there is no evidence to show that slight spoilage is deleterious to health or is injurious to the food value of fish flesh. The amount of spoilage permissible must be determined by its effect on the odour, appearance, and palatibility of the fish flesh. Finally all fish must undergo such organoleptic tests. If our estimations happen to correspond with

those of most people, we might successfully use these tests to differentiate good fish from bad. Kimura and Kumakura (1934) consider fish fresh if it contains less than 10 mg. % ammonia and amino nitrogen, and in the first stages of decomposition if it contains more than 30 mg. %. Lücke and Gerdel (1935) found in all types of fish, values of 20 mg. % volatile basic nitrogen. They claim fish is not objectionable up to 30 mg. %. Glassmann and Rochwarger (1929) gave 20 mg. % ammonia nitrogen as the limit for good fish, while Tillmans and Otto (1924), Yamamura (1933), and Tanikawa (1935) gave 30 mg. % volatile basic nitrogen as the upper limit. While these conclusions are influenced by the standards of the examiners and by differences in analytical procedures, it would appear that the maximum increase permissible is about 10 to 20 mg. % volatile basic nitrogen. The maximum range in variation found in fresh cod muscle is 7 mg. %. Later amounts are, therefore, to that extent without significance, which would lead one to conclude that volatile basic nitrogen as a test for freshness is little better than organoleptic tests.

TRIMETHYLAMINE AS A MEASURE OF SPOILAGE

Trimethylamine oxide occurs in marine animals (Hoppe-Seyler and Schmidt 1928, Yoshimura and Fujise 1929, and Hoppe-Seyler 1930). Poller and Linneweh (1926), who isolated it from herring muscle and roe, state that bacteria quickly reduce it to trimethylamine, which accounts for the large amount of the latter in herring brines.

Dreyer (unpub.) and later Boury and Schvinte (1935), the latter using Okolov's (1932) method, found that trimethylamine increases as fish spoil. One of us (S.A.B.) on the basis of experiments made in 1929 concluded that the marked increase in volatile bases in brines of slack-salted fish just before and during spoilage consisted chiefly of trimethylamine. Ammonia, as shown by Nessler's reagent, made up only a small part of the increase. As Nesslerizations were clear and matched standard ammonium sulphate, dimethylamine, which precipitates Nessler's reagent and monomethylamine, which produces a greenish-yellow colour, could have been present only in very small amounts. The increase in the volatile bases in slack-salted fish not affected by Nessler's solution, which occurs coincident with rapid bacterial increase, suggested the possibility of the utilization of trimethylamine values as a measure of freshness in untreated fish.

For trimethylamine, as for total volatile bases, to be effective in measuring spoilage: (1) its amount must be determined rapidly enough to provide whatever number of determinations are needed to make the results significant; (2) its increase must agree closely with bacterial increase; (3) odours should appear when it has reached a given value; (4) its production by autolysis should be negligible; (5) its original variability must be negligible in comparison with its increase.

Analytical Methods

The first attempt to fractionate the volatile bases into an ammonium fraction and a fraction supposedly trimethylamine was made by determining first the

total base by titration of a distillate, and the ammonia by Nesslerization of a second distillate. The difference between the two values is the trimethylamine fraction. Boury and Schvinte (1935) found that the volatile amines, determined in this manner, and trimethylamine, determined after the destruction of ammonia and other amines by nitrous acid, agreed very closely.

Because of the time-consuming nature of the titration Nesslerization procedure, and because of the considerable error in the determination of very small traces of trimethylamine as found in absolutely fresh fish, other methods were adopted for the determination of both trimethylamine and total volatile bases. The reaction between formaldehyde and ammonia was utilized to hold back the ammonia while the trimethylamine was distilled over. Earlier experiments were performed with the modified Parnas still, but a modification of the method of Conway and Byrne (1933) for the determination of ammonia was found to be sufficiently accurate for our purpose and so easy and rapid of manipulation that it was adopted. Because this method is not adapted to the use of semi-solid material, muscle press juice was used instead of ground muscle. The press cup was of brass and was perforated with holes 1/16 inches (ca. 2.1 mm.) in diameter. The cup was lined with Whatman no. 1 filter paper and filled with the muscle under examination. A circular sheet of filter paper was placed over the sample, a brass plunger was inserted and pressure applied. The time required from the mincing of the muscle until the juice was treated with formaldehyde as described below was approximately 10 minutes. In the inner ring of the Conway dish is placed an excess of standard acid, usually about seventieth normal. In the outer ring is placed 1 ml. press juice and 0.5 ml. formaldehyde solution. Usually commercial formalin, shaken with an excess of magnesium carbonate and filtered or decanted gives a zero or an extremely low blank. If the blank is appreciable, the formalin may be distilled from an acid solution after which the volatile acids distilling over may be neutralized, preferably with basic magnesium carbonate. After the addition of the formaldehyde the cover is placed on the Conway dish and the dish is rotated to ensure thorough mixing of press juice and formaldehyde. The cover is partially removed, 1 ml. saturated potassium carbonate is added from a very fast delivery pipette and the lid is again slid into place. The dish is rotated to mix the contents and is placed in an incubator at 36° C. for 2 hours, after which the excess standard acid is back titrated with standard alkali, using Conway and Byrne's (1933) modification of Tashiro's methyl red methylene blue indicator.

This fraction of the volatile bases of fish muscle may not be entirely trimethylamine. This question is under investigation at the present time. But, realizing that we may be in error, we are referring to it as trimethylamine for the sake of brevity.

The accuracy of the method has not been determined. Trimethylamine can be recovered from solutions of trimethylamine and ammonia within 5 per cent. Trimethylamine oxide in solution, in approximately the concentration as found in cod muscle is hydrolysed by the alkali during the incubation period, producing results approximately 0.02 mg. per cent too high. In table III is shown the recovery of volatile base not reacting with formaldehyde, from fresh and spoiling cod muscle press juice. With fresh cod muscle press juice it would appear that

there is some hydrolysis during the analytical procedure. In spoiling muscle press juice, the amount of hydrolysis is less than the errors in analytical procedure. In either case the errors are not sufficiently great to affect the usefulness of the method.

TABLE III. Time required for complete distillation of trimethylamine nitrogen from cod muscle press juice at 36° C.

Time (min.)	Mg. nitrogen per 100 ml. distilled from fresh press juice	Mg. nitrogen per 100 ml. distilled from partially spoiled press juice
30	0.04	0.95
	0.03	0.95
60	0.09	1.25
	0.11	1.29
90	0.15	1.43
	0.16	....
120	0.18	1.66
	0.17	1.65
	0.17	....
150	....	1.71
	....	1.65
	....	1.61
180	0.21	1.61
	0.21	1.63

The speed of the modified Conway method is adequate. An analyst and one assistant can prepare samples, isolate press juice and determine the trimethylamine fraction at the rate of about twelve per hour.

RELATION TO BACTERIAL POPULATION

Bacteria and trimethylamine have not been compared directly to see whether or not they increase simultaneously. That they do so may be deduced from two facts. Total volatile bases and bacteria increase simultaneously, as shown in figure 2. Figure 3 shows that both ammonia and trimethylamine increase rapidly during the same period and that the increase in trimethylamine is greater than that in ammonia.

RELATION TO SPOILAGE ODOURS

In our trimethylamine experiments, as in those with volatile bases, there was considerable disagreement among observers as to the first appearance of odours in spoiling fish. The sensitivity of the observer, the temperature of the fish, its freedom from confinement, all influence the strength of the odour. Therefore,

the relationship between smell and trimethylamine value must be rather indefinite. The consensus of opinion is that odours appear first at about 4 to 6 mg. trimethylamine nitrogen per 100 ml. press juice, at 10 mg. they are definite, and at 20 to 30 mg. they are strong.

Effect of Autolysis

The effect of autolysis on the production of trimethylamine has been determined both in muscle tissue and in muscle press juice. Because the Conway

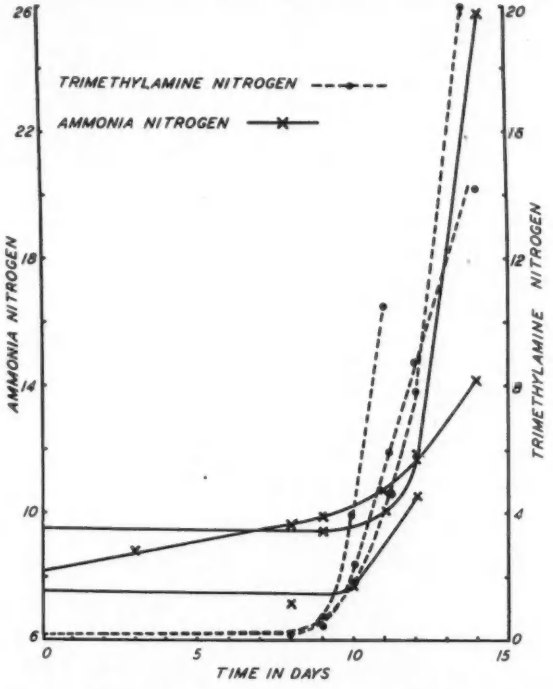

Figure 3. The increase in ammonia nitrogen and trimethylamine nitrogen in three commercia cod fillets at 0.5° C., expressed in mg. per 100 ml. press juice.

dishes cannot be used for the determination of trimethylamine in tissue, and because it is extremely difficult to obtain sufficiently large samples of sterile muscle to isolate 1 ml. of press juice, the production of trimethylamine in sterile muscle tissue was followed by means of the original distillation-titration Nesslerization method. The results are shown in table IV. It will be recalled that in this method the trimethylamine nitrogen is obtained by subtracting the Nessler value from the total bases obtained by titration with standard acid. In some cases, as a result of experimental error, the Nessler value exceeded the titration value. These are shown as negative values in table IV. They are inserted as such to indicate the approximate limit of accuracy of the method. All changes

TABLE IV. Trimethylamine production in sterile cod muscle

Fish no.	Temp. 0° C.			Temp. 20° C.		
	Time (days)	N_2 Mg/100 gm.		Time (days)	N_2 Mg/100 gm.	
		Original	Final		Original	Final
1	22	0.3	0.2	5	0.3	−0.3
2	16	0.2	0.0	5	0.2	−0.3
3	22	−0.1	0.2	5	0.2	0.6
4	22	0.2	0.5	5	0.2	0.3
5	22	−0.2	0.0	11	−0.2	−0.5
6	22	−0.1	0.1	11	−0.1	0.4

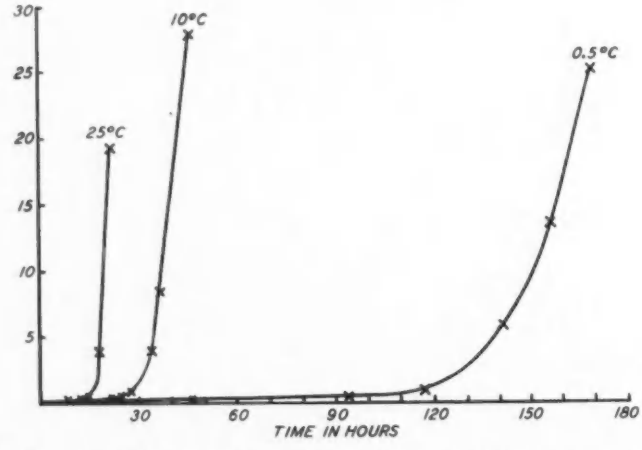

FIGURE 4. The rate of trimethylamine production in mg. per 100 ml. non-sterile cod muscle press juice at 25°, 10°, and 0.5° C.

TABLE V. Trimethylamine production in sterile cod muscle press juice

Time (days)	Temp.	Trimethylamine N_2 Mg./100 ml.
0	10° C.	0.19
2	10° C.	0.19
12	10° C.	0.17
30	10° C.	0.48
0	0° C.	0.15
8	0° C.	0.15
30	0° C.	0.21

in the level of trimethylamine are less than the experimental error of about 0.5 mg. % and no rise in trimethylamine could be demonstrated in sterile tissue by this method. The experiment was repeated, using press juice filtered through a Seitz filter, and the modified Conway analytical procedure. The results are shown in table V. The rate of trimethylamine production in contaminated press juice at 25° C., 10° C. and 0.5° C. is shown in figure 4 and the ammonia and trimethylamine development in commercial fillets was shown in figure 3. There is undoubtedly a very small post-mortem production of trimethylamine not associated with bacterial action, but this production is negligible in comparison with that resulting from the action of bacteria during the spoilage of commercial fish or fillets.

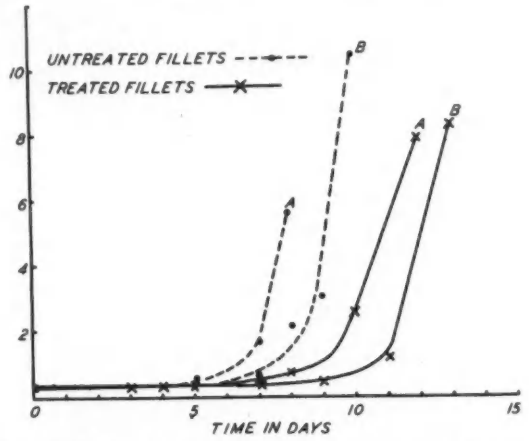

FIGURE 5. The increase in trimethylamine nitrogen in mg. per 100 ml. press juice in commercial cod fillets with and without borates as preservatives. A and B indicate different preservatives.

ORIGINAL VALUES

The lowest value found for trimethylamine nitrogen in more than 100 individual fish (cod, haddock, herring, hake and pollock) was 0.06 mg. per 100 ml. muscle press juice and the highest 0.30 mg., the average being 0.17 mg. Odours appear at about 4 mg., and are definite at about 10 mg. Hence the rise to the first appearance of odours is almost twenty times the maximum variation among individuals, and is about twenty times the average original value.

EFFECT OF PRESERVATIVES

Where the regulations concerning their use permit, preservatives are sometimes employed to delay the onset of spoilage. Since the history of fish may not always be available, a method for the determination of spoilage which functions properly on preserved as well as on untreated fish would be advantageous. The method was tried with two of the commonest borate preservatives. Each preservative was tried on 15 fillets, the opposite fillet of each fish being untreated and used as control. The fillets were stored at 0.5° C., and samples were taken

from each at intervals for analysis. The average time required to reach a level of 4 mg. % trimethylamine nitrogen was 2.5 days and 2.6 days longer than in the case of untreated controls. The spoilage curves of typical treated and untreated fillets are shown in figure 5. The only significant difference between the treated and the untreated fillets is the lengthening of the latent period. After the trimethylamine starts to rise, the increase is about equally rapid in both treated and untreated fish.

Discussion

The trimethylamine value of fish muscle fulfils all the conditions required of an effective test for freshness. By means of the concentration of trimethylamine nitrogen one is able to express quantitatively the condition of the fish. In so far as the authors are aware, this is the only measurement of reasonable speed and accuracy more sensitive than the sense of smell. As shown in figures 3 and 5, a measurable rise in fish stored at 0° C. occurs about two days before the threshold of smell is reached. But unfortunately it is still impossible to forecast keeping time outside this narrow time limit. Figures 3 and 5 show a latent period, which we have found to vary from five to twelve days, in which no measurable amount of trimethylamine is produced. During the whole of this period one can determine only that the fish is still quite fresh.

SUMMARY

Volatile bases can be used as a measure of spoilage for cod only if the original value of the fish in question is known. The rise in volatile bases coincides with the increase in bacteria, autolysis does not play a significant role, the production of volatile bases correlates with organoleptic tests, but the variation in original values may be as great as the difference between absolutely fresh and slightly spoiled fish.

A method has been developed which fractionates the volatile bases into two parts, one consisting of ammonia and possibly a very small amount of monomethylamine, and the other consisting of all the other volatile bases in the fish muscle. The second fraction is almost negligible in fresh fish but increases rapidly during spoilage. This fraction is believed at present to consist mainly of trimethylamine resulting from the breakdown of trimethylamine oxide. The trimethylamine fraction rises rapidly during the onset of spoilage, may be correlated indirectly with the bacterial increase, has a negligible increase due to autolysis, and its original concentration is sufficiently low and sufficiently constant to make the rise during the onset of spoilage significant. Because of a slight hydrolysis resulting from the analytical procedure, the original values are not true values but possibly 0.1 mg. % too high. This error appears constant. The method has been proven to measure the degree of spoilage accurately in fish preserved with borates as well as in untreated fish.

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